IOWA STATE COLLEGE
JOURNAL OF SCIENCE

A Quarterly of Research

VOL. IX
1934-1935

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No. 1, October, 1934

No. 2, January, 1935

No. 3, April, 1935

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PHYSIOLOGY OF LACTOBACILLUS FRUCTIVORANS SP. NOV., ISOLATED FROM SPOILED SALAD DRESSING[1]

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During an outbreak of spoilage in commercially prepared salad dressing, the only organism that was isolated proved to be a species of Lactobacillus. The evidence pointed to this lactic acid bacillus as responsible for the spoilage. Although this report will deal principally with the physiology and classification of *Lactobacillus fructivorans* sp. nov., some remarks concerning the spoilage may be of interest.

The commercial preparation of mayonnaise and various types of salad dressings has become an important food industry in the United States. Mayonnaise is an emulsion composed chiefly of vegetable oil, egg yolk, water, and vinegar to which certain condiments are added. The composition of this product is subject to certain restrictions under food laws. Other salad dressings are made from similar ingredients to which a variety of substances such as pickles, olives, meat, sugar and starch may be added. There do not appear to be definite standards in regard to the composition or method of preparation and preservation of these types of salad dressings. Large numbers of bacteria which may be present in the ingredients are practically all destroyed by heating during the manufacture of the dressing. Bacterial spoilage by any surviving organisms in the finished product does not usually occur owing to the concentration of acetic or lactic acid employed. Other undesirable changes in salad dressings, such as discolorations, changes in consistency, emulsion break-down, and especially the development of rancidity present difficulties to the manufacturers.

The literature relating to bacterial spoilage of salad dressings is not extensive. Pederson (1930) studied an outbreak of spoilage due to an aerobic spore forming organism. Spoilage due to similar types of bacteria had been reported by other investigators and their findings were reviewed by Pederson.

THE FERMENTED SALAD DRESSING

Samples of spoiled salad dressing were submitted for examination. The manufacturer was inexperienced with this type of product, and the spoilage was excessive; about one-fifth of the first five thousand cases were returned as undergoing spoilage. The flavor and odor of the spoiled product were not abnormal; the reaction was found to be pH 4.1, which is not different from that to be expected in this type of product. A slight lumpiness was present. The development of gas in the product, the only evidence of spoilage, took place very slowly. On opening the glass con-

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tainers a "bubbling over" of a portion of the contents occurred. No marked gas pressure was developed by any of the fermented samples.

Stained preparations of the spoiled product revealed slender gram-positive rods occurring in chains. Morphologically the organisms were of one type; they were not uniformly distributed, but were present primarily in clusters of intertwined chains. No spores were observed.

ISOLATION AND STUDY

Attempted isolations of the causative organism were at first unsuccessful. Aerobic and anaerobic platings on milk powder agar, veal infusion agar and plain agar showed no growth. Subsequent platings from two samples were made on tomato juice agar (ordinary nutrient agar containing about 20 per cent of neutralized tomato juice). The number of colonies gave a plate count of about 50,000 bacteria per gram of spoiled salad dressing. Growth was much slower and there were fewer colonies on the aerobic plates than on those incubated in an atmosphere containing carbon dioxide. The difficulty experienced in obtaining growth on ordinary media followed by the successful use of tomato juice agar is substantially the experience of Mickle and Breed (1925) in isolating *Lactobacillus lycopersici* from spoiled catsup. These authors state, ". . . little success attended efforts of isolation with autolysed yeast broths, sodium oleate media, casein digest or milk powder agars, . . . even though the hydrogen ion concentration was varied. . ." The isolated organisms were of one type, being gram-positive, non-spore forming rods. In cultural behavior, particularly in forming large entire subsurface colonies and distinctly dome-shaped surface colonies, the organisms more closely resembled certain of the propionic acid bacteria than the lactobacilli. Other interesting characteristics were noted which suggested the advisability of a detailed study.

DESCRIPTION OF LACTOBACILLUS FRUCTIVORANS sp. nov.

In the following description, the broth, agar, and gelatin media contained 20 per cent of neutralized filtered tomato juice unless otherwise stated.

MORPHOLOGY

Grown on agar at 30° C. in an atmosphere to which approximately 50 per cent carbon dioxide is added the rods are from 0.4 to 0.8μ by 1.5 to 4μ. The chains of cells show a distinct "looping" characteristic, often turning upon themselves. The individual cells may also be curved. (Fig. 1.) When the organism is grown aerobically, this turning characteristic is much more pronounced so that the cells occur principally in tightly massed groups (Fig. 4). In broth the cells occur as slender rods in chains and the swelling and curving tendencies are not pronounced. Long filaments occur frequently. The cells are non-motile, stain evenly and are gram-positive.

CULTURAL CHARACTERS

Agar slant. Aerobic growth is scanty. Heavy inoculation leads to a beaded white growth. (Fig. 8b.) Under anaerobic conditions at 30° C. an abundant growth is obtained within 48 hours. Equal volumes of air

and nitrogen or air and carbon dioxide allow good development. The growth is smooth, white, butyrous and opaque.

Agar stab and agar shake. There is an optimum zone of growth in the vicinity of 2 cm. below the surface (Fig. 9a). When not crowded the colonies in an agar shake may attain a diameter of 4 mm. In glucose tomato agar shake culture splitting of the agar due to gas development usually appears, but is never extensive.

Agar colonies. Surface colonies are circular with a diameter from 1 to 3 mm., after 4 days at 30° C., in a 50 per cent CO_2 atmosphere. The colonies are raised, smooth, white, amorphous and entire. In a humid atmosphere with a slightly moist agar surface the colony edge may be filamentous (a young colony is shown in fig. 6). On a dry surface, the edge is entire and the colony was be "dome-shaped." The sub-surface colonies are lens-shaped and entire.

Gelatin. No liquefaction.

Broth. In broth, brought to a boil before inoculation, there is a "snowflake" type of growth to within one centimeter of the surface. This growth settles to the bottom or adheres to the wall of the tube (Fig. 10c). If the broth is not boiled before inoculation quite a different cultural characteristic is observed, the growth develops more slowly and is confined at the bottom to a depth of about 5 mm. The supernatant liquid is clear.

PHYSIOLOGICAL CHARACTERS

Gas production. Glucose broth fermentation in Smith tubes leads to the development of gas amounting to about 10 per cent of the closed arm after 7 days at 30° C.

Litmus milk. The organism remains alive for some time and there may be slow growth. Milk remains unchanged. The addition of levulose or tomato juice leads to coagulation of the milk.

Oxygen relationship. Microaerophilic.

Catalase production. Negative.

Temperature relationships. Optimum 25° to 30° C., growth at 37° C., no growth at 40° C. The cells die rapidly at 65° C.

Dissimilation of carbohydrates. Yeast extract 0.2 per cent, peptone 0.5 per cent, K_2HPO_4, 0.1 per cent, neutralized tomato juice 5 per cent, carbohydrate 1 per cent. Acid from glucose and levulose.

No acid from: Mannose, galactose, sucrose, maltose, lactose, trehalose, xylose, arabinose, α-methyl glucoside, inulin, dextrin, glycerol, raffinose, salicin, starch, dulcitol, sorbitol.

Considering the foregoing description, as well as the dissimilative characteristics reported in the following pages, which show that lactic acid but no propionic acid is produced from glucose, the organism should be placed in the genus Lactobacillus. It is not like any of the described species and is distinctive in that with the exception of glucose and levulose it fails to ferment the commonly employed carbohydrates. Levulose is much more strongly attacked than glucose.

Lactobacillus fructivorans is suggested as a name to designate this new species because of its preference for fruit sugar.

OBSERVATIONS OF THE NUTRITIVE REQUIREMENTS OF L. FRUCTIVORANS

The organism does not grow perceptibly in peptone or in yeast extract broth; upon addition of tomato juice good growth is obtained. Although no special attempt was made to determine the constituent in tomato juice responsible for this stimulation of growth some observations with tomato juice and other vegetable and plant extracts may be of interest.

Pure unneutralized tomato juice supports a luxuriant growth of the organism and as low as two per cent of tomato juice in peptone broth is stimulating. Tomato juice which has been fermented with *Aerobacter aerogenes,* filtered and re-sterilized, still retains the growth promoting property.

The juices of apples and pears added to peptone broth constitute satisfactory media, although they are not as stimulating as tomato juice. Aqueous extracts of carrot, cabbage, soy bean and potato were added to peptone broth, but none exhibited an appreciable stimulation of *L. fructivorans.* Similar results were obtained with 0.5 per cent concentrations of the sodium salts of malic, citric and tartaric acids in peptone broth.

Difficulty was encountered in the choice of a medium for study of the physiological characteristics of *L. fructivorans.* The importance of a proper nitrogen source in such studies has been stressed by Orla-Jensen (1919) and by Sadler and Eagles (1932). Undoubtedly this is an important consideration, for apparently inconsistent results may be obtained by changing the nitrogen source. However, in the case of certain lactobacilli, a preference for certain types of carbohydrates has been observed. In this connection Müller-Thurgau and Osterwalder (1912) have shown that with *Bact. gracile,* if glucose and malic acid are present in a given medium, the malic acid is utilized first, apparently in preference to the glucose. Certain bacteria are known to show a predeliction for levulose; this preference is further illustrated in the present case. In a suitable medium, glucose is fermented, yet in glucose-peptone broth no appreciable growth or fermentation of the sugar occurs. On the other hand, peptone broth containing levulose is a satisfactory medium, although improved upon the addition of tomato juice. Müller-Thurgau (1903) reported similar observations when employing a related species, *Bact. mannitopoeum.* He found that upon the addition of various carbohydrates and salts of organic acids to a basal medium of peptone, 1 per cent; K_2HPO_4, 0.2 percent; and NaCl 0.5 per cent, that growth occurred only in the presence of sucrose or levulose. There was no growth in the presence of glucose or maltose even though these sugars are fermented by *Bact. mannitopoeum* when the basal medium contains yeast extract and malic acid. From these observations, and from some results in the studies reported below, it appears that the "mannitol forming" organisms in the genus Lactobacillus exhibit a predelcition for levulose, and in one case were found to utilize an organic acid in preference to glucose.

COMPARATIVE STUDIES ON L. FRUCTIVORANS AND L. GRACILIS

Lactobacillus fructivorans belongs to the group of mannitol-forming bacteria reported in studies on spoiled wines by various investigators.

Pederson (1929) has studied organisms representing a number of species of Lactobacillus. The only previously described species which resembles *L. fructivorans* is one described by Müller-Thurgau (1908), as *Bacterium gracile*. Further description of *Bact. gracile* was recorded by Müller-Thurgau and Osterwalder in 191 and 1917. Additional studies on *Bact. gracile* have not appeared, although the organism is referred to as *L. gracile* in Bergey's Manual (1930). *Lactobacillus gracilis* is the correct form.

Our appreciation is expressed to Dr. Osterwalder for sending us a culture of *L. gracilis*.

L. fructivorans and *L. gracilis* differ in size (compare the preparations from agar and broth shown in figures 1 and 3; 5 and 7). The looping tendency of the chains of cells is characteristic of both. On morphological grounds, *L. gracilis* should belong to the genus Leuconostoc as described by Hucker and Pederson (1931), and the original description of the organism does not conflict with this allocation.

Culturally, the two species are similar. The same type of milk-white colony is characteristic of both. The oxygen relationships, nutritive requirements, and temperature relationships of the two species are similar. The colonies of *L. fructivorans* are as a rule larger, a difference in structure, apparent only under the microscope (Figs. 2 and 6), is related to the differences in cell size. This fact may partly explain the differences in broth cultures (Fig. 10).

Both species produce inactive lactic acid.

The two organisms may be differentiated on the basis of fermentation reactions. Trehalose and α-methyl glucoside are fermented by *L. gracilis* but not by *L. fructivorans*.

PRODUCTS OF DISSIMILATION OF GLUCOSE AND LEVULOSE
QUANTITATIVE METHODS FOR DETERMINING PRODUCTS OF FERMENTATION

Lactic Acid: An aliquot of the fermented liquor was evaporated to 10 cc., acidified to congo-red paper and taken up in anhydrous sodium sulfate. The resulting dry crumbly mixture was placed in a thimble and continuously extracted for eight hours. The ether was distilled off and the residue brought to a volume of 100 cc. with water. A 10 cc. fraction was neutralized to phenolphthalein with sodium hydroxide, boiled three minutes with an excess of alkali and again brought to neutrality. The lactic acid was then determined by the method of Friedemann and Kendall (1929).

Ethyl alcohol was determined by the method described by Stahly, Osburn, and Werkman (1934).

Acetic acid was determined by steam distilling and partitioning the distillate with ethyl ether according to the method of Osburn, Wood, and Werkman (1933).

Carbon dioxide was caught in a Bowen potash bulb and weighed.

Mannitol was formed only in solutions containing levulose. Levulose is soluble and mannitol insoluble in ether, making possible the separation from interfering substances by ether extraction.

The residue from the ether extraction was extracted eight hours with 95 per cent alcohol. The alcohol was evaporated and the mannitol dissolved in water and made up to 100 cc. The determination was made by the method suggested by Smit (1914).

Qualitatively, the products obtained from the fermentation of glucose by both species were the same, i .e., ethyl alcohol, lactic acid, acetic acid, and carbon dioxide. These products and in addition mannitol were formed from levulose.

For the quantitative determination of the products of fermentation a medium consisting of one per cent peptone, five per cent tomato juice, 0.2 per cent potassium phosphate (dibasic) and two per cent sugar was prepared. One liter of the medium was placed in two-liter Erlenmeyer flasks. The sugar was sterilized separately and added aseptically. Ten cubic centimeters of a five day culture of the organism in question were used as inoculum. All fermentations were incubated at 30° C. for 21 days. During fermentation, nitrogen was continuously bubbled through the medium.

In order to compensate, in a measure, for the acid contained in the tomato juice added to the medium, the yields obtained by analysis of an uninoculated flask were subtracted from those obtained in the fermented medium.

Table 1 shows the yields of products in the anaerobic fermentation of glucose and levulose by the two species. No propionic, succinic, or formic acid was found in any of the fermentations.

The data in table 1 emphasize the utilization of levulose. A review of the literature indicates that this is characteristic of the lactic acid organisms producing mannitol from levulose. Levulose functions as both hydrogen acceptor and (after splitting) as donator.

It is seen that *L. fructivorans* is more active in fermenting glucose and levulose than *L. gracilis*. The former fermented 38.5 millimols of glucose and 113.8 millimols of levulose while *L. gracilis* fermented only 10 millimols of glucose and 89.3 millimols of levulose. The difference in the quantity of sugar fermented by the two species may, in part, be due to a difference in medium required for optimal growth.

It has been noted that these organisms are micro-aerophilic. This fact is further emphasized by an experiment with the same medium as was used above. Air passing first through a bead tower containing potassium hydroxide, was bubbled through the medium for five hours at the beginning of the fermentation to remove carbon dioxide present in the system. It was again aerated after two weeks and at the conclusion of the fermentation which continued thirty days. The results of this experiment are presented in table 2.

Comparison of aerobic and anaerobic fermentations shows that greater utilization of glucose takes place under aerobic conditions. This, in part, may be due to the longer period of fermentation. The greater portion of fermentation takes place, however, in the first few days as shown by Weinstein and Rettger (1932) and Fred, Peterson and Davenport (1920). The longer period of fermentation is not likely to influence the sugar utilization to the extent shown by the data in tables 1 and 2.

The results show that *L. fructivorans* is a hetero-fermentative lactic acid organism and is related to those described by Pederson (1929), Fred, Peterson and co-workers (1919, 1921), Gayon and Dubourg (1901), von Steenberg (1920), and Müller-Thurgau and Osterwalder (1912).

TABLE 1.　*Anaerobic dissimilation of glucose and levulose by L. gracilis and L. fructivorans*

| | L. fructivorans | | | | L. gracilis | | | |
| | Glucose | | Levulose | | Glucose | | Levulose | |
	Milli-mols	Percent-age of sugar	Milli-mols	Percent-age of sugar	Milli-mols	Percent-age of sugar	Milli-mols	Percent-age of sugar
Sugar used	38.5	......	113.8	......	10.0	......	89.3	......
Ethyl alcohol	37.8	32.7	6.8	2.0	9.0	30.0	13.4	5.0
Carbon dioxide	67.8	29.3	52.4	7.7	14.7	24.5	35.8	6.7
Acetic acid	15.4	13.3	42.9	12.7	9.2	30.7	47.0	17.5
Lactic acid	12.25	16.2	12.6	5.5	1.5	7.5	7.3	4.1
Mannitol	....	....	84.5	74.5	....	....	61.5	68.8
Percentage of sugar accounted for		91.5		102.4		92.7		102.1

TABLE 2.　*Aerobic dissimilation of glucose by L. fructivorans and L. gracilis*

| | L. fructivorans | | L. gracilis | |
	Millimols	Percentage of carbon in sugar	Millimols	Percentage of carbon in sugar
Sugar used	47.55	......	57.3	......
Ethyl alcohol	40.8	28.6	48.2	27.5
Carbon dioxide	26.7	9.4	20.0	5.7
Acetic acid	12.0	8.4	11.6	6.0
Lactic acid	34.5	36.1	25.7	22.0
Percentage of sugar accounted for		82.5		60.2

SUMMARY

Latobacillus fructivorans sp. nov., isolated from spoiled salad dressing, is described and compared with *L. gracilis* from which it was morphologically and culturally differentiated.

L. fructivorans and *L. gracilis* belong to the group of heterofermentative lactic acid bacteria. Dissimilation of glucose leads to the production of inactive lactic acid, acetic acid, ethyl alcohol, and CO_2 and in addition mannitol is formed from levulose.

Photo-micrographs of *L. gracilis (Bact. gracile)* agree with those in the original descriptions and indicate that it is of the Leuconostoc type and not a species of Lactobacillus as described by Bergey (1930).

Dissimilation of levulose by both species differs from that of glucose in that the nature and quantitative relationships of the reduced products are changed. Part of the levulose acts as a hydrogen acceptor to be reduced to mannitol. The reduction of levulose is compensated for by greatly lowered yields of other reduction products, that is, ethyl alcohol.

The mannitol-forming members of the genus Lactobacillus are not readily differentiated from species of Leuconostoc Hucker and Pederson (1931). The two types act similarly in the dissimilation of glucose and levulose and agree in other respects, such as in cultural appearance, temperature requirements, and habitat. Since the cells of some Luconostoc species may elongate into a rod the morphological distinction cannot be maintained.

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PLATE I

Fig. 1. *Lactobacillus fructivorans* grown on tomato juice agar at 30° C. in an at-
mosphere of 50 per cent carbon dioxide. Gram stain x 1080.

Fig. 2. Six day colony of *L. gracilis* on tomato juice agar at 30° C. and in an at-
mosphere of 50 per cent carbon dioxide. Unstained. x 60.

Fig. 3. *L. gracilis* grown on tomato juice agar at 30° C. in an atmosphere of 50
per cent carbon dioxide. Gram stain x 1080.

Fig. 4. *L. fructivorans* grown aerobically on tomato juice agar at 30° C. Eight day
culture. Nigrosine preparation. x 1080.

Fig. 5. *L. gracilis* grown in tomato juice broth at 30° C. Gram stain x 1080.

Fig. 6. Six day colony of *L. fructivorans* on tomato juice agar at 30° C. and in an
atmosphere of 50 per cent carbon dioxide. Unstained. x 60.

Fig. 7. *L. fructivorans* grown in tomato juice at 30° C. Six day culture (then held
two days at room temperature). Nigrosine preparation. x 1080.

Fig. 8. a. *L. gracilis* in glucose tomato juice agar at 30° C. in an atmosphere of 50
per cent carbon dioxide. Unstained.

b. *L. fructivorans* as under a.

Fig. 9. a. *L. gracilis* in glucose tomato juice agar at 30° C. Showing two growth
zones. Six day cultures.

b. *L. fructivorans* in glucose tomato juice agar at 30° C. Six day culture.
Showing growth zone.

Fig. 10. a. *L. gracilis* in tomato juice broth at 30° C. Six day culture.

b. Uninoculated control tube of tomato juice broth.

c. *L. fructivorans* in tomato juice broth at 30° C. Six day culture. Showing
growth zone and also precipitated organisms

PLATE I
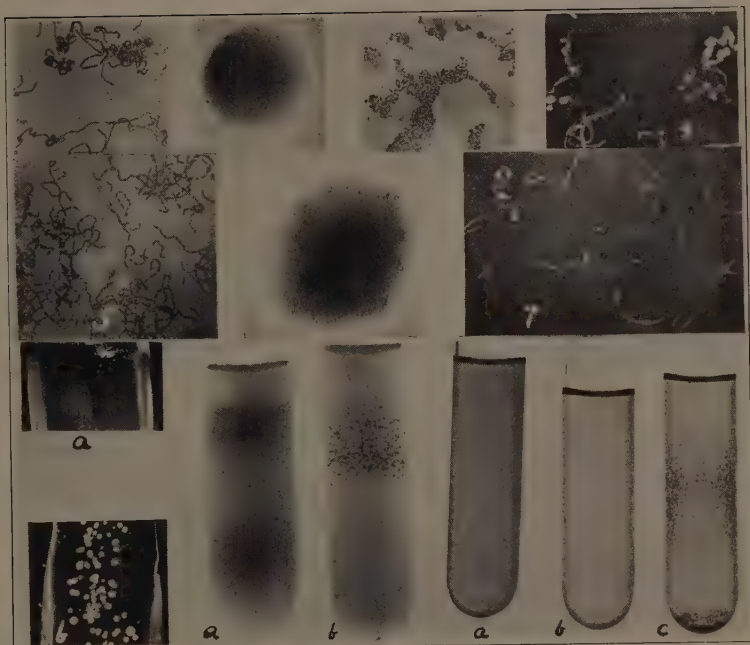
ON THE TOTAL BLOOD (HEMOLYMPH) CELL COUNT OF THE FIELD CRICKET, GRYLLUS ASSIMILIS PENNSYLVANICUS BURM.[1]

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Total blood cell counts of vertebrates, particularly of mammals, have been reported frequently in the literature, usually in connection with physiological investigations. On the other hand, total blood cell counts of invertebrates, especially of insects, appear to have been recorded very infrequently. Hardy (1892), using a Gower's hemacytometer, obtained an average total count of 286 cells per mm³ of blood for the crustacean, Astacus, the values ranging from 250 to 400 cells per mm³. Blood was used both fresh and after osmic acid vapor fixation. Tillyard (1917) roughly estimated that an entire dragonfly larva of very small size must contain only two or three hundred cells, and suggested that the number may be increased with each succeeding ecdysis. Haber (1926) estimated that there are about 1,400 to 2,500 cells per medium sized drop of blood from the cockroach, *Blattella germanica* Linn. Landois and Landois (1865) state that "by general estimation" the blood cells of the larva of *Smerinthus populi* remain at a constant level, reaching their highest count shortly before the pupal stage and subsequently decreasing to a minimum during metamorphosis; they do not present figures for total cell counts. Hollande (1930) states that Aghar (1928)[2], in his doctorate thesis, presents values for insect total blood cell counts, finding respectively 12,000 and 10,000 cells per mm³ of blood from *Pieris brassicae* L. and *Aporia crataegi* L. Yeager and Tauber (1932) obtained an average total cell count of approximately 30,000 cells per mm³ of blood from the roach, *Periplaneta fuliginosa* Serville, individual counts ranging from 16,916 to 65,355 cells per mm³ blood. They also report (1933) an average total cell count of about 30,000 cells per mm³ blood from the roach *Periplaneta orientalis* L., the values ranging from 14,400 to 57,600. Fischer (1934) found the range for total blood cell counts for *P. orientalis* to be from about 9,000 to 84,000 cells per mm³ of blood, with an average of approximately 34,000.

The present paper contains an analysis of 220 total blood cell counts obtained from the common field cricket, *Gryllus assimilis pennsylvanicus* Burm.

ANIMALS

The field crickets used for the total blood cell counts reported here were randomly collected at Ames, Iowa, in the months of June, July, September, October, and November; that is, from their first appearance in the spring until their disappearance in the fall. Only the larger adults

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[2]The authors have not had access to Aghar's original publication.

and nymphs were used for the counts, which were made as soon after collection as practicable. While kept in the laboratory the crickets were supplied with water and food (banana, oat sprouts, and grass). Specimens collected in spring and summer appeared to remain in good condition in the laboratory, but many, especially the adults, caught in the late fall often died after being caged indoors for a few days. Whether this mortality was due to natural seasonal causes is not known, but since the spring animals could be kept for several weeks in good condition, this factor probably was the important one in producing the high death rate in the late fall.

METHOD

Counts recorded here were obtained with a double chamber hemacytometer, customarily used for making total blood cell counts of vertebrates. A specially made diluting pipette, by means of which 1.22 mm³ of blood could be diluted 150 times, was employed in order that a minute quantity of the rapidly coagulating blood of the insect might be quickly diluted and cell coagulation thereby prevented. The dilution fluid consisted of 0.081 M NaCl, 0.002 M KCl, 0.001 M $CaCl_2$, 0.005 per cent gentian violet, and 0.125 per cent glacial acetic acid. Otherwise, the procedure was the same as that usually employed with vertebrate blood.

The blood sample was obtained from an antenna, cut off near its base.

All but six of the animals collected in September, October and November were subjected to glacial acetic acid vapor to prevent cell coagulation (Shull, Riley, Richardson, 1932). An animal so treated was suspended by a thread for five to ten minutes, depending on the reactions of the animal, above the surface of 25 cc. of glacial acetic acid in a closed 250 cc. wide mouth bottle. The other animals were employed without acetic acid treatment.

RESULTS

Total blood cell counts have been obtained from 220 different individuals of *Gryllus assimilis pennsylvanicus* Burm. The values are widely distributed with a mean of 70,118 and a range from approximately 15,000 to 275,000 cells per mm³ of blood. It is not necessary to tabulate here the individual values of the counts since the data can be better illustrated by the frequency distributions employed in the analysis of these results.

When the counts are arranged according to the sex or stage of development of the animals from which they were obtained, the various group averages are as follows: mature and nymphal males (73 animals), 64,020; mature and nymphal females (147 animals), 73,147; mature males and females (197 animals), 73,099; nymphs of both sexes (23 animals), 44,589; mature males (63 animals), 69,886; mature females (134 animals), 74,609; male nymphs (10 animals), 27,064; female nymphs (13 animals), 58,071. It is seen that the average for all females is higher than the average for all males; that the average for the mature animals is higher than the average for the nymphs; that the average for the mature females is higher than that of the mature males; and that the average for the nymphal females is considerably higher than that of the nymphal males. These figures indicate that, in general, the average total blood cell count for the female crickets tends to be higher than that for the males, although the modes for the two sexes coincide (Fig. 2).

ANALYSIS

If the individual values of the 220 total blood cell counts are grouped with class intervals of 20,000 cells per mm[3] of blood, their skewed frequency distribution is that shown by the polygons of figure 1, A. With this grouping, the distribution is very nearly that represented by Pearson's Type III curve (see Elderton, 1906) as indicated by the values of the criteria β_1, β_2, κ, provided that the single value of 275,490 be discarded (a procedure justified by Chauvenet's criterion)[3]. From an experimental standpoint, a class interval of 20,000 cells per mm[3] of blood is too large to be of much practical use, for it implies too great a counting error; also, its size tends to obscure certain characteristics of the distribution that appear with a 10,000 cells per mm[3] class interval. Nevertheless, it is of interest that the distribution from 10,000 to 110,000 is almost symmetrical, with nearly normal distribution.
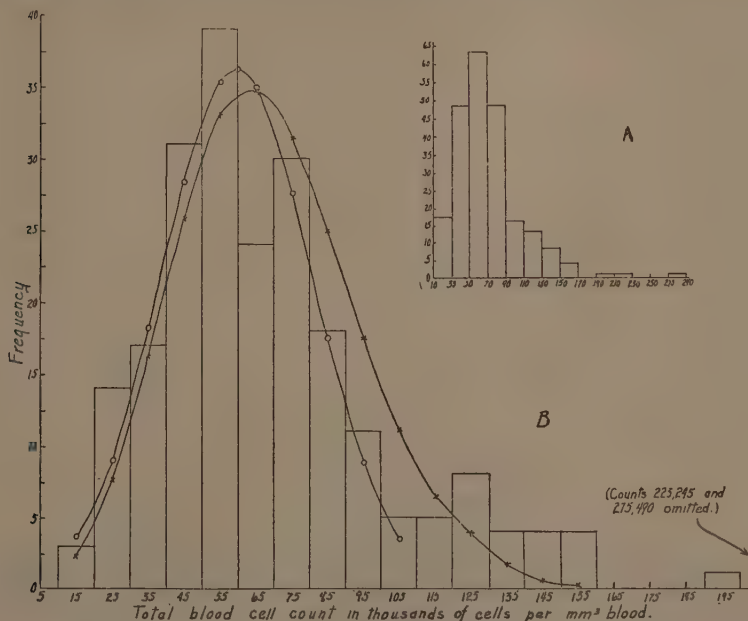
Fig. 1. A—Frequency distribution of 220 total blood (hemolymph) cell counts of the field cricket, *Gryllus assimilis pennsylvanicus*, with class interval of 20,000 cells per mm[3] of blood. B—Frequency distribution of the same series of counts but with a class interval of 10,000. The average for the series is 70,118 cells per mm[3] of blood. The line connecting the circles is a theoretical normal distribution curve calculated for all counts of value 110,000 or less. The line connecting the crosses is a theoretical Poisson series curve calculated for the series of 218 counts of value 200,000 cells per mm[3] of blood or less. The polygons represent the observed distribution.

In figure 1, B, a class interval of 10,000 cells per mm³ of blood is used. The nearly perfect symmetry of the 10,000 to 110,000 range is broken partly by the low frequency of the 65,000 polygon. With that exception, this limited range tends to be normally distributed, as is shown by the moderately good fit of the theoretical normal (Type VII) curve (circles), calculated on the basis of only those counts of 110,000 cells per mm³ or less. It is obvious, however, that the entire distribution is not one of pure type but is a complex one, skewed toward the higher counts and with modes at 55,000, 75,000, and 125,000 cells per mm³. [In figure 1, B, the crosses represent a Poisson series (see R. A. Fischer, p. 55) calculated for 218 counts of 200,000 cells per mm.³ or less, using a mean of 63,394. Although the theoretical values, in general, follow the form of the observed distribution, it is obvious that the fit is not entirely satisfactory.] The question arises, what are the factors tending to produce this trimodality and to skew the distribution of these counts? While a complete answer to this question must await further investigation, interesting leads may be obtained by additional analysis of the data.
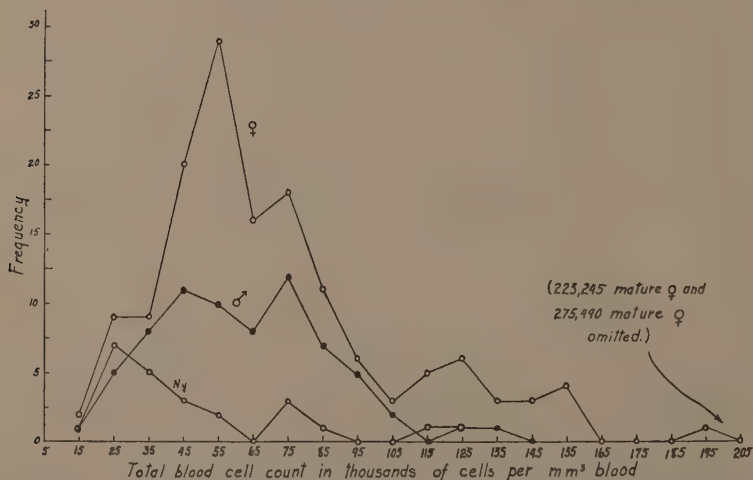
Fig. 2. Distribution of total blood cell counts among (♀) mature and nymphal females, (♂) mature and nymphal males, and (Ny) male and female nymphs. The two highest counts of 223,245 and 275,490 cells per mm³ of blood (both mature females) are omitted. The means are 73,147 (♀), 64,020 (♂), and 44,589 (Ny).

The entire series of counts may be divided into three groups: counts from females (nymphs and adults), counts from males (nymphs and adults), and counts from nymphs of both sexes taken together. The frequency distributions for the three groups are shown in figure 2. Each distribution appears as a skewed, trimodal curve, of the same type as that of the whole series (Fig. 1, B), with modes at 55,000, 75,000, and 125,000 (females); at 45,000, 75,000, and 125,000 (males); and at 25,000, 75,000 and 115,000 (nymphs) cells per mm³. From this it would appear that the trimodality and asymmetry of the entire series (Fig. 1, B) are not due to

differences of distribution in male and female nymphs, males and females; or in other words, that they are not due to maleness, femaleness, or a general growth factor (difference between nymphal and imaginal stages). It is of interest, however, that all counts above 120,000 cells per mm³ were obtained from mature insects. Furthermore, all counts between 120,000 and 145,000 are from either mature males or mature females, and all the remaining figures above 145,000 cells per mm³ of blood are from mature female crickets alone. These high counts in the distributions of the two sexes, particularly in the female, contribute largely to the asymmetry of the entire series. Also, the difference between the maximal limits of the counts from nymphs and adults may contribute, partially, to the existence of the skewness of the entire distribution, but the number of nymphs counted in this study is small (23) and does not warrant definite conclusions.
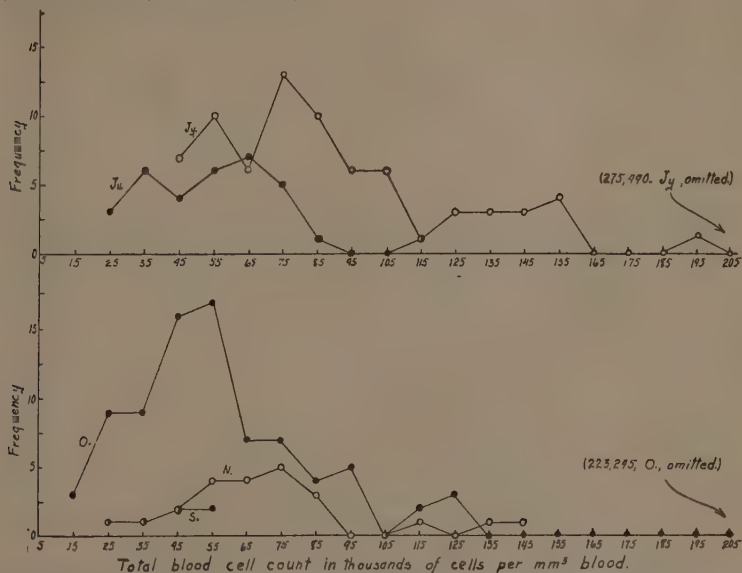
Fig. 3. Distributions of total blood cell counts among animals collected in June (Ju.), July (Jy.), September (S.), October (O.), and November (N.). The means are 73,073 (June), 83,710 (July), 59,552 (September), 60,144 (October), and 71,039 (November). The two highest counts (223,245 and 275,490) are omitted.

In a similar way, the total series of counts may be grouped according to the month during which the animals were collected. Figure 3 shows the frequency distributions of five groups of crickets collected in the months of June, July, September, October, and November, respectively. With the exception of September, during which only a few animals were collected, the monthly frequency distributions are trimodal, skewed toward the higher counts, and, hence, essentially like the distribution of the entire series (Fig. 1, B). The values at the various modes are shown in figure 3. The modes at 25,000, 95,000, and 115,000 may be of question-

able significance. From this it is seen that the trimodality and skewness of the entire distribution are also characteristic of the monthly frequency distributions and, consequently, it would appear that these properties of the entire distribution (Fig. 1, B) are not due to collecting the animals at various times of the year.

The number of mature males, mature females, and male and female nymphs collected in the different months are as follows:

	June	July	Sept.	Oct.	Nov.
Males	19	31	2	10	1
Females	7	41	4	69	13
Nymphs	7	1	0	6	9

The frequency distributions of counts from acetic acid treated animals are shown in figure 4. The counts from the acid treated crickets tend to be somewhat lower than those from normal, untreated animals, the former group having an average of 57,735 cells per mm^3 of blood and modes at 55,000, 75,000, and 125,000, while the counts from the untreated animals are distributed with an average of 82,380 cells per mm^3 and with modes at 55,000 and 75,000; both distributions are skewed toward the higher counts. The authors have found from experience that in making insect total blood cell counts, the greater the degree of blood coagulation the greater is the number of cells lost to the cell coagulum and the fewer are the cells in the counting chamber, and hence the lower is the total cell count. Fixation of the cells by heat or acetic acid vapor prevents the formation of a cell coagulum, prevents the loss of cells from the counting chamber, and prevents the total cell count from appearing less than its true value, in so far as cell coagulation is concerned. If the use of acetic acid vapor were to affect the distribution, it would be expected to make the counts somewhat higher than those from the untreated animals. The distributions in figure 4, however, show the opposite effect; also, it would seem very probable that the acid is not a factor producing the trimodality and skewness of the entire distribution (Fig. 1, B). This conclusion is supported by the work of Fischer (1934), who found that the use of acetic acid vapor as an anticoagulant results in total blood cell counts significantly higher than counts made on blood from normal, untreated animals, and who also noted that in the latter cases there was always evidence of cell coagulation in the counting chamber.[4]

It has been suggested by Allard (1929) that some crickets may have more than one brood per annum, some animals overwintering in the egg stage, some in the nymphal stage. If this is the case, in the species used in this study, the large nymphs and imagos collected in June and July would represent one brood (overwintering in the nymphal stage) while

[4]In the present work, clumps of agglutinated cells appeared rarely in the counting chamber when blood from normal, untreated animals was used; this is due to the fact that the authors employed a specially made micro diluting pipette, the use of which permits very rapid dilution of only 1.22 mm^3 of blood and thereby prevents the occurrence of cell coagulation. If, as infrequently occurred, evidence of cell coagulation was noted in the pipette or the counting chamber, the preparation was discarded and the count not made. The low counts obtained by Fischer from untreated animals are very probably due to the use of an ordinary white blood cell diluting pipette, which, requiring a larger amount of blood, permits the occurrence of cell coagulation.

those captured in September, October, and November would represent another brood (overwintering in the egg stage). Examination of the frequency distributions for June, July, October, and November (Fig. 3) shows that they all tend to be trimodal and asymmetrical toward the
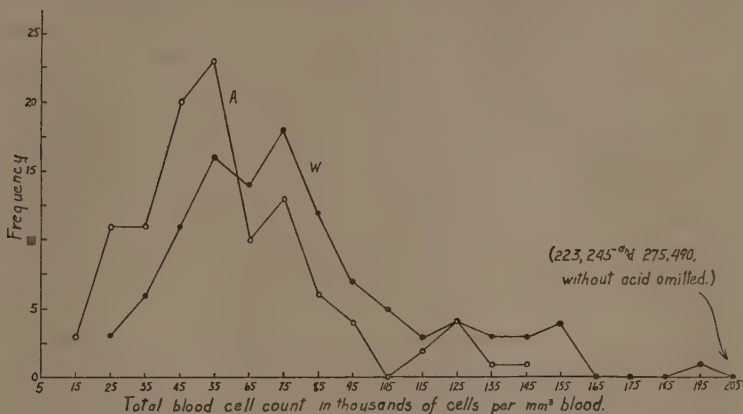
Fig. 4. Distribution of total blood cell counts among (A) animals treated with acetic acid vapor as anticoagulant and (W) normal, untreated animals. The means are 57,735 (A) and 82,380 (W) cells per mm³ of blood. This difference is not to be considered due to the use of acid. Compare with Fig. 5. See text. The two highest counts (223,245 and 275,490) are omitted.
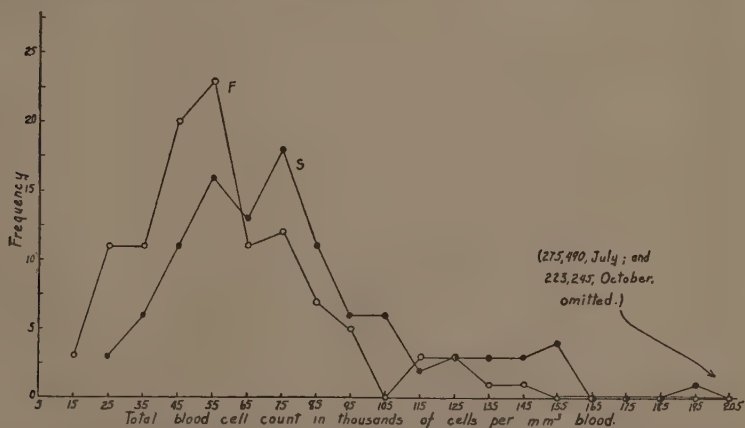
Fig. 5. Distributions of total blood cell counts among animals collected in (S) early summer (June-July) and (F) fall (September-October-November). The means are 79,479 (S) and 61,414 (F) cells per mm³ of blood. Compare with Fig. 4.

higher counts, thereby resembling the entire distribution (Fig. 1, B), whose characteristics of trimodality and skewness, therefore, are not attributable to the month during which the animals were collected.

However, if the counts from animals collected in June and July are grouped together and separated from the group of animals made up of those collected in September, October, and November, the distributions of the two groups take the form shown in figure 5. The mean for the June-July group is 79,479; for the September-October-November group, 61,414. The difference between the two means is 18,065 with a standard error of ± 4,780. This difference is significant since it is more than twice its standard error (see R. A. Fischer, p. 101 and p. 105). The June-July and the September-October-November distributions are similar, respectively, to the distributions from normal, untreated animals and from acetic acid vapor treated animals (Fig. 4), due to the fact that all but six of the fall animals were treated with acid vapor before making the count. The question therefore arises, do the *means* of the June-July and September-October-November distributions differ because of the use of acetic acid vapor or because of other factors related to the season during which the animals were collected, as, for example, to the existence of two different seasonal broods? It has already been indicated that such a difference is contrary to the expected effect of the use of acetic acid vapor as an anticoagulant. This greatly increases the probability that the difference between the two means may be due to the local existence of two different broods of this cricket, even though the two broods would seem to be similar with respect to the general characteristics of their total blood cell count distributions.

DISCUSSION

The analysis of counts just given indicates that the skewness and trimodality of the entire frequency distribution are not due to maleness, femaleness, general growth stage (i. e., difference between nymph and imago), to the use of acetic acid vapor as an anti-coagulant, to the month during which the animals were collected, or to the existence of two different seasonal broods of this cricket. Although the distributions of the counts from males, females, and nymphs all possess a common characteristic in being skewed toward the higher counts (Fig. 2), the counts above 145,000 cells per mm^3 of blood, all of which were obtained from mature female crickets, make the asymmetry of the entire distribution more prominent (Fig. 1, B).

Usually nothing was known of the instar, proximity to molting or to oviposition periods, or to other special physiological and pathological conditions which might occur in a given animal at the time of observation, but in several cases a few records of this kind were obtained, as indicated below:

Animal	Total count (Cells/mm³ blood)	Record of condition
No. 489♀	223,245	Parasitized, *Gordius* larvae
No. 488♀	125,080	” ” ”
No. 350♀	106,493	Just after molting
No. 367♂	120,825	During molt
No. 266♀	118,328	Just before molting (1½ hrs.)
No. 418♀	199,995	Ovipositing
No. 433♀	275,490	”

In none of the cases above is the total blood cell count low. On the basis of these observations, it may be tentatively suggested that the skewness of the entire frequency distribution toward the higher counts is perhaps

due in part to the occurrence of excessively high total blood cell counts in animals that are in certain physiological or pathological states brought about by conditions such as ecdysis, oviposition, and parasitism. Although the possibility of this suggestion is not lessened by the fact that the frequency distribution tends to be symmetrical in the range from 10,000 to 110,000 cells per mm³ of blood, the proof depends upon further investigation definitely showing the relationship between total blood cell count and ecdysis, oviposition, parasitism, or other special physiological or pathological conditions of the animal. Tillyard (1917) has suggested that the number of blood corpuscles in a dragonfly larva appeared to increase with each succeeding ecdysis; and Landois and Landois (1865) have stated that the number of blood cells in the larva of *Smerinthus populi* varied at different stages in this insect's life history.

Normally there are several circulatory and nutritional factors which would tend to increase the range of distribution of insect total blood cell counts. (1) The insect possesses an open type of circulatory system in which the blood flows into large sinuses and hemocoelic cavities and, presumably, comes into direct contact with most of the animal's tissues. Circulation may be sluggish, and it is not improbable that cells would settle out of the slow-moving streams. Moseley (1871), while studying the circulation in the wings of *Blatta orientalis,* noted that "the corpuscles attach themselves to the inner wall of the vessel." Yeager and Hendrickson (1934), who refer to the preexisting literature on the subject, have recently observed the flow of blood in the wing veins of the roach *Periplaneta americana* L., and have noted that a portion of the blood cells in these channels may, at times, temporarily cease to flow. Likewise, Viallanes (1882) noted a similar phenomenon in the circulating blood of a transparent fly larva. In their paper, Yeager and Hendrickson suggest that the temporary cessation of corpuscular flow, especially if extended to other blood channels of the body, would tend to produce a variation in total blood cell count, provided the percentage of such non-circulating cells is of sufficient variability. It is not improbable that this factor may have affected these cricket counts. (2) It is not impossible that differences in the state of nutrition of individual insects (the amount of water ingested, contained in, or given out from the animal's body) may also have affected the total count by causing a dilution or a concentration of the circulating blood. Haber (1926) reports that if specimens of *Blattella germanica* L. are fed dry food in a dry habitat the animals soon reach a state in which it becomes difficult to obtain blood from them; well fed individuals, on the other hand, contain more blood and bleed easily. Moseley (1871) observed that if specimens of *Blatta orientalis* were deprived of food and water for several days, circulation in the insect would become very feeble or almost absent. Muttkowski (1923), working with Leptinotarsa larvae, found that the animals appeared turgid after feeding due to the increased volume of blood plasma which distended the hemocoel. If starved, the same larvae become flaccid and wrinkled due to the decrease of blood plasma in the body. Berlese (1901), in discussing insect metabolism, pointed out the relationship which exists between the digesting food in the alimentary canal and the plasma surrounding it and noted the changes in the plasma as the digested material entered the blood. The entrance of this food material undoubtedly would alter the volume of blood in the body of the animal. Wigglesworth (1931, p.

425) noted an increased amount of hemolymph in the bug, *Rhodnius prolixus,* following the ingestion of rabbit blood. Miall and Denny (1886), in discussing the blood of *P. orientalis,* state (p. 142), "The quantity varies greatly, according to the nutrition of the individual; after a few days' starvation, nearly all the blood is absorbed." Other workers, including Newport (1845) and Bruntz (1908), also stated that the blood volume of an insect may vary according to the nutritional condition of the individual. These variations in volume probably bring about changes in the concentration of corpuscles by concentrating or diluting the blood. (3) It has been shown that in the cricket (Yeager and Knight, 1933) as well as in the roach (Yeager, Shull, and Farrar, 1932) blood coagulation involves the formation of a cell coagulum. It is not impossible that cell coagulation, at the point of sampling (antenna), may account partially for the wide range of total count values for the cricket, although the authors believe such an effect to be negligible or very slight, since preparations were discarded whenever signs of cell coagulation appeared in the diluting pipette or the counting chamber. Furthermore, figure 4 shows that counts from normal animals are slightly higher than those from acid vapor treated animals whose blood was not in a coagulable state. Nevertheless, the very low counts given in the literature by Hardy (1892) for Astacus blood, and by Haber (1926) for *Blattella (Periplaneta) germanica* blood may well have been due in part to loss of cells by coagulation since these authors apparently used no effective anti-coagulant measures.

SUMMARY AND CONCLUSIONS

(1) Two hundred and twenty total blood (hemolymph) cell counts of the field cricket, *Gryllus assimilis pennsylvanicus* Burm, have been obtained by a slight (micro) modification of the usual hemacytometer technique.

(2) The entire series (220 counts) has a trimodal frequency distribution, skewed toward the higher counts (class intervals of 10,000 cells per mm^3 of blood).

(3) The skewness and trimodality of the entire series apparently are not due to maleness, to femaleness, to general growth (that is, difference between nymph and imago), to the month during which the animals were collected, to the existence of two broods per annum that overwinter, respectively, in the egg and nymphal stages, or to the anticoagulant action of glacial acetic acid vapor.

(4) It is suggested that the skewenss may result, at least partially, from the occurrence of excessively high total blood cell counts accompanying certain physiological and pathological conditions such as ecdysis, oviposition, and parasitism; and possibly, in part, to certain circulatory factors inherent in the animal. The counts of 145,000 cells per mm^3 and higher were obtained from female crickets (Fig. 2). These tend to accentuate the asymmetry of the entire distribution (Fig. 1, B).

(5) The total blood cell counts of this cricket are widely distributed, ranging from about 15,000 to approximately 275,000 cells per mm^3 of blood, with an average of 70,118 cells per mm^3. Certain circulatory and nutritional factors characteristic of the insects may be responsible, in part, for the wide range of counts.

(6) In general, the average count from imagos is higher than that from nymphs.

(7) The average total blood cell counts from June-July crickets and from September-October-November crickets differ significantly; it is tentatively suggested that the difference may be due to the local existence of two seasonal broods within this species.

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NOTES ON THE BIOLOGY AND CONTROL OF NEOSCIARA OCELLARIS (COMSTOCK) (DIPTERA, SCIARIDAE)[1]

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Formerly the root gnats were included in the Mycetophilidae as a subfamily, Sciarinae, but recently taxonomists have accorded them family rank. The harmful effects caused by the feeding of their larvae on the underground portions of various plants were mentioned by early American entomologists; however, these injuries did not attract much attention in greenhouses and mushroom cellars until more recently. Many of the species of Sciaridae feed on the healthy tissues of higher plants, while, so far as is known, the Mycetophilidae feed exclusively on fungi or decaying organic matter. The primary purpose of these investigations was to study the biology of one species, *Neosciara ocellaris* (Comstock), common in greenhouses about Ames, Iowa. During the investigations it was found that the maggots were causing considerable damage to greenhouse plants, and thus some control measures were undertaken.

Neosciara ocellaris was found to be abundant in greenhouses at Ames, Iowa. Specimens have also been collected at Washington, D. C., and Lancaster and Buffalo, New York. It is not unlikely that the species occurs in every state, because of the ease with which the immature stages could be carried in the soil about the roots of nursery plants.

HISTORY AND IMPORTANCE

Walsh (1868) seems to have been the first writer to note that maggots of some species of sciarids were associated with a form of potato scab. In 1894 Hopkins made similar observations, and assigned to the insect which caused the damage the scientific name, *Epidapus scabiei;* however, the fly is now placed in the genus Pnyxia. Chittenden (1901) reported that maggots of Sciara were causing considerable damage to roots of potted plants, lettuce, cucumbers, and carnations, and Johannsen (1912) also found the maggots damaging roots of wheat, corn, potatoes, and other plants. In 1916 Hungerford published his studies of the biology of *Neosciara coprophila* (Lintner). He found the maggots of this species to be causing considerable damage to plants in conservatory windows and to potted plants. Gui (1933) reported that maggots of *Pnyxia scabiei* caused injury to potatoes in several localities in Ohio during the years 1926 to date (1933).

As far as the writer is aware, *Neosciara ocellaris* has not been mentioned in the literature as an injurious species, but it is not unlikely that it has many times been confused with *Neosciara coprophila,* a species

which it closely resembles. The adult flies of *N. ocellaris* usually are not noticeably abundant in greenhouses in Iowa during the summer, but when cool weather appears they increase in numbers in the greenhouse benches, and sometimes the maggots can be turned out of the soil in squirming masses. Under favorable conditions these flies increase rapidly, and the maggots cause considerable damage which first becomes apparent when the plants lose their healthy, vigorous growth.

LIFE HISTORY

Technique. The rearing records were obtained under constant conditions at 25° C. and nearly 100 per cent relative humidity. The larvae were reared individually in stender dishes with close fitting tops, pea leaves being used as food. In order to maintain sufficient moisture, the bottom of each stender dish was covered with a moist disc of ordinary paper toweling; this light background also aided in locating the cast head capsule. Each larva was examined twice daily to determine the time of ecdysis, these observations being made about 8:00 A. M. and 4:00 P. M. The width of the head capsule and the approximate length of the body was determined after each ecdysis.

Eggs. The eggs are oval in shape, 0.25 mm. in length and 0.09 mm. in width; the cephalic ends are slightly broader than the posterior ends. When first deposited they are pale greenish-yellow in color, but they soon change to a pearly white. During the third day of incubation the head of the developing embryo begins to darken and shows at the cephalic end as a spot with indefinite outline. The head of the embryo becomes quite dark and conspicuous before hatching, and at this time the larva is active within the chorion. The eggs are laid in irregular clusters of 3 to 40. They are usually placed in crevices in the soil or just below the surface of the soil along the stems of plants. Under greenhouse conditions the eggs usually hatch in four to five days; however, the incubation period may be only three and one-half days when kept at 30° C. and nearly 100 per cent relative humidity. The minimum temperature to which the ova were exposed was 20° C. and the maximum was 34° C.; under the former conditions the ova hatched in seven days, but under the latter conditions the embryos failed to develop. The eggs will not hatch unless they are in contact with moisture, or under conditions where the relative humidity is nearly 100 per cent. The larva escapes from the egg by eating a small hole in the cephalic end of the chorion, and if food is available it begins to feed immediately.

Larva. The larva is characterized by a strongly sclerotized, shining black head. It is 12-segmented, footless, more or less cylindrical, slightly tapering from the middle towards both ends, soft, translucent, and of a whitish color. There are eight pairs of conspicuous spiracles located along the sides. Although the larval life was greatly lengthened as a consequence of starving and of subjecting them to other adverse conditions, the number of instars was always four.

As there are no pronounced external morphological changes during larval development, it is not necessary to give a separate description of the larva in the various stages. The width of the head capsule and the approximate length of the body of the larva in the various stadia are given in table 1.

TABLE 1. *Results of observations on the width of the head capsule and the length of the body of N. ocellaris larvae*

Instar	Number of observations	Mean width head capsule mm.	Mean width of body mm.
1st	50	0.08	0.65
2nd	50	0.13	2.00
3rd	50	0.21	4.00
4th	50	0.30	5.5

Although the head capsule of a first instar larva is dark and sclerotized, it is never so strongly sclerotized nor so shiny-black as that of the older larva. There is a distinct increase in the width of the head capsule after each ecdysis, but no increase between molts, so the width of the head is an excellent character to use in determining the various instars.

There is no observable period of quiescence before ecdysis, except at pupation time. At the first, second, and third ecdysis the head capsule and outer body cuticula separate immediately posterior to the head and the head capsule splits along the median ventral line, the larva then crawls out of the old body covering, but at the last ecdysis, the head capsule splits along the median dorsal line, the head capsule and outer covering of the body separate as mentioned before. The process of molting requires only a few minutes, and it occurs wherever the larva happens to be feeding. Immediately after ecdysis the head is translucent, as is the remainder of the body. However, it soon begins to show pigment, and within a period of two to four hours it is again strongly pigmented. Although larvae have the head sufficiently hardened to feed immediately after hatching, newly molted larvae cannot begin to feed until the head becomes sclerotized.

Twenty of the larvae reared in these experiments (Table 2) were placed on fresh pea leaves and kept at a constant temperature of 25° C., and approximately 100 per cent relative humidity. The maximum time from egg to adult under these conditions was 25 days, and the minimum time was 21 days, the average being 23 days. Thirty larvae (Table 3) were kept under the same temperature and humidity conditions, but they were placed on leaves that were previously allowed partly to decay. The maximum time from egg to adult in this group was 25 days and minimum 18 days; the average being 19 days. There was, therefore, a difference of 4 days in the length of the larval life when fed on this material at different stages of freshness.

The maggots of *Neosciara ocellaris* are practically omnivorous in their feeding habits. The writer has observed them feeding on the decaying bodies of pupae and adults of their own kind, and on the roots and underground stems of the following plants: geranium, nasturtium, pea, potato, corn, grass, wheat, rape, lettuce, cucumber, and carnation. They were found also in great numbers in decaying onion bulbs, but it is not likely that they attack the healthy tissues. Even in soil in which plants are not growing, the maggots will develop apparently normally if there is an abundance of manure or other organic matter. The maggots as a

rule begin to feed first on the root hairs, gradually working inward to the larger roots and even to the underground stems. The writer has examined peas in which Neosciara maggots had eaten the root hairs and smaller roots, stripped the outer cambium from the larger roots and had even made extensive tunnels into the underground stems. The injury to roots is often severe enough to cause a sudden wilting and death to seedling plants, a result quite similar to that caused by certain seedbed diseases such as damping-off, and it is not unlikely that the injury resulting from the two causes are sometimes confused.

TABLE 2. *Length of life stages of N. ocellaris when fed on fresh pea leaves**

Individual number	Egg	First instar	Second instar	Third instar	Fourth instar	Pupal stage	Total
1	4	5	2	3	6	4	24
2	4	5	3	2	5	4	23
3	4	5	2	2	4	4	21
4	4	4	3	2	6	4	23
5	4	5	2	2	5	4	22
6	4	5	2	dead			
7	4	5	2	2	4	4	21
8	4	5	2	2	5	4	22
9	4	5	2	2	4	4	21
10	4	5	1	2	6	4	22
11	4	5	dead				
12	4	5	2	2	5	4	22
13	4	5	2	2	5	4	22
14	4	5	2	2	6	4	23
15	4	4	5	2	6	4	25
16	4	4	5	2	5	4	24
17	4	5	2	2	4	4	21
18	4	6	2	3	5	5	25
19	4	5	2	2	5	4	22
20	4	7	2	2	6	4	25
Mean	4	5	2.5	2	5	4	23

*Stated in days and at 25° C.

The maggots from *Neosciara ocellaris* are very susceptible to drying, and when the soil in which they are feeding becomes dry they are soon inactive, and unless moisture is added, death invariably results. If several maggots are feeding in close vicinity of each other they congregate in a group, when the soil becomes dry, and secrete a mucilaginous substance that for a short period tends to prevent desiccation.

The larva discontinues feeding about a day before it pupates. At the time when feeding ceases the compound eyes of the developing fly show above as two dark pigmented areas immediately posterior to the larval head capsule. With the aid of a microscope it can be seen that the body now contains a large amount of conspicuous fat bodies. Immediately after the larva stops feeding it commences to construct a silken chamber in which it later is to pupate. As construction proceeds, the pupal chamber becomes shorter and thicker and when completed is approximately 3 mm. in length and 1 mm. in width. Soon after the pupal chamber is

completed, which usually requires two days, the larva becomes quiescent and normally pupates within a few hours.

Pupa. Female pupae average about 2 mm. in length; the male pupae are slightly smaller. The legs are folded against the breast and venter, and the antennae are bent around the compound eyes and extend between the wings and legs. There are six abdominal and two thoracic spiracles; the prothoracic spiracles being located above the wing base, immediately behind the antennae. The pupa is white immediately after pupation, but it gradually becomes darker, and before emergence it has
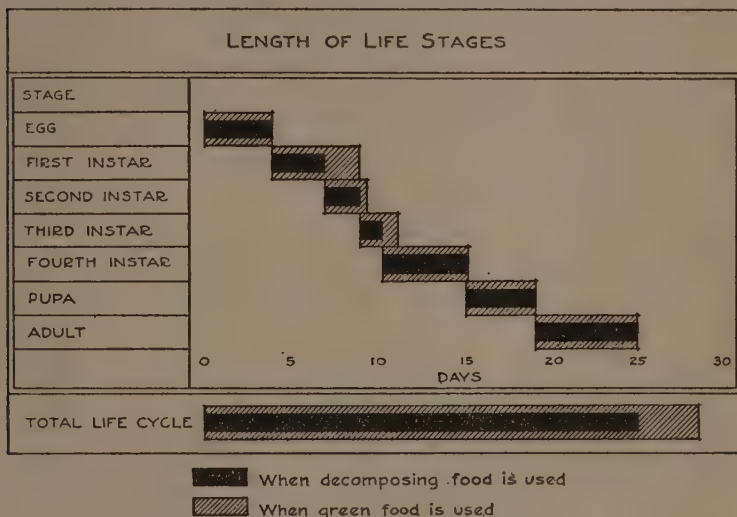
Fig. 1. Showing length of life stages of *Neosciara ocellaris*.

acquired the color pattern of the adult. As is shown in tables 2 and 3, the 50 larvae reared, with the exception of two, emerged on the fourth day after pupation, thus indicating that the length of the pupal stage is quite constant under these conditions.

A few hours before the adult is to emerge, the pupa usually works its way to the surface of the soil. This is accomplished by squirming motions in which, no doubt, the free appendages play an important role. In heavily infested greenhouses many pupae and pupal skins can be found with their posterior ends fastened to the soil and their anterior ends projecting into the air at about a 60 degree angle. Immediately before emergence, the adults can be seen squirming vigorously about in the pupal skin, which suddenly develops a T-shaped rupture. The transverse anterior part of the T-shaped opening, as is characteristic in other Orthorrhapha, is between the head and thorax and the stem of the T extends down the mid-dorsal line for about one-fourth the length of the abdomen. It requires only a few moments for the adult to work its way through this small opening. Then it remains inactive for a short time, allowing the wings to unfold and the body to become hardened. The

TABLE 3. *Length of life stages of N. ocellaris when fed on decomposing pea leaves**

Individual number	Egg	First instar	Second instar	Third instar	Fourth instar	Pupal stage	Total
1	4	3	2	2	6	4	21
2	4	3	2	2	6	4	21
3	4	3	2	1	4	4	18
4	4	3	2	1	4	4	18
5	4	4	2	1	3	4	18
6	4	3	2	1	4	4	18
7	7	3	2	2	5	4	20
8	4	3	2	1	6	4	20
9	4	4	2	1	3	5	19
10	4	3	2	1	4	dead	
11	4	3	2	1	6	4	20
12	4	3	2	1	5	4	19
13	4	3	2	1	5	4	19
14	4	3	2	1	4	4	18
15	4	4	2	1	10	4	25
16	4	3	2	2	5	4	20
17	4	3	2	2	5	4	20
18	4	2	2	2	4	4	18
19	4	3	2	1	4	4	18
20	4	3	2	1	8	4	22
21	4	3	2	1	4	4	18
22	4	3	2	2	5	4	26
23	4	3	2	1	5	4	19
24	4	3	3	1	5	4	20
25	4	2	2	1	4	4	18
26	4	3	2	1	5	4	19
27	4	3	2	1	5	4	19
28	4	3	2	1	4	4	18
29	4	3	2	2	4	4	19
30	4	3	2	1	5	4	19
Mean	4	3	2	1	5	4	19

*Stated in days and at 25° C.

abdomen of a recently emerged female is greatly distended by the large number of eggs it contains.

Adult. Length of dried male specimens 1.5 to 2 mm.; width from tip-to-tip of wings 3.75 mm. Head black, antennae dark brown, less than three-fourths length of body, composed of 12 segments plus 2 subsegments; tarsal joints yellowish brown; pronotum light yellowish-brown; mesonotum yellowish in the center and darker at the edges; scutellum dusky brown; metathorax dark brown, almost black; abdomen with caudal portion of the segments blackish, cephalic portions yellowish-brown; claspers lighter brown; halteres light brown at base, the knobs blackish. Tibiae and tarsi dusky brown; femors paler, coxae still paler. Wings grayish, R_1 and R_s dark, prominent, M_1 and M_2 not prominent; petiole of M indistinct; costa extending more than one-half distance from R_s to M_1; R_1 ending near apex of wing; base of R_s distad of the midpoint between the humeral cross-vein and the tip of R_1; petiole of Cu short, less than one-half as long as basal section of M_1. Claspers on the

dorsal-mesal margin with 2 or 3 strong setae in addition to 5 or 6 finer, apical ones, hypopygium with no median ventral lobe at base.

Length of dried female specimens 2 mm. to 2.5 mm.; width from tip-to-tip of wings 4.5 mm. Coloration same as for male except abdomen may show more yellow between segments, especially in gravid females where the abdomen is greatly distended, thus exposing more of the inter-segmental area. Wing venation same as for male.

The captive adults used in these experiments were fed on a 10 per cent sucrose solution, on which they lived for about the same length of time as adults under more natural conditions, the life-span being about a week. Mating usually occurs soon after the flies have emerged; coition generally taking only a few minutes, but sometimes lasting five to ten minutes. The male approaches the female from behind, and with the claspers, which are opened and closed spasmodically, the end of the female abdomen is grasped and then the male turns over to face in the opposite direction to that of the female, thus bringing the genitalia into juxtaposition. The species is both polygamous and polyandrous.

Females sometimes begin to oviposit late in the same day in which they emerge, but normally oviposition occurs during the second and third day. Some females lay all of their eggs in one day, but usually deposition extends over a period of two or three days. Twenty-five recently emerged females were kept individually in containers and the number of eggs laid by each determined. The maximum number laid by an individual was 175 and the minimum 123; the average being 140.

HABITS

The writer has watched the adults in the greenhouse feeding on the ooze that is found around decaying manure or other decaying organic matter, and so far as he is able to determine, they do not take any other under natural conditions. As a consequence of the feebleness of flight and their minute size, the adults usually escape the notice of all except the careful observer. Their color, which closely resembles that of the soil, and their secluded habits tend to make them rather inconspicuous. The flies apparently avoid the direct rays of the sun, and are found most abundantly in the shaded parts of greenhouses. Soil that is high in decaying organic matter and moist and shaded, seems to be ideal for their activities. The flies are strictly diurnal and do not become active until about eight o'clock in the morning, at which time they can be seen crawling from beneath particles of soil, vegetation, and out of crevices. The adults are strong runners but very weak flyers, often resorting to running to escape danger. A characteristic action of the males is to run hurriedly over the surface of the soil with their wings and antennae vibrating continuously. Although the females are rather active, they are not nearly so active as the males.

UNISEXUAL PROGENIES

While conducting life history experiments with *Neosciara ocellaris*, the writer found that the progeny from a given female are preponderantly of one sex. As these flies are polygamous, one male can be mated to several females with the result that some of the females give rise to female

progeny and others to male progeny, thus it can be shown experimentally that the factor or factors determining sex of progeny is held by the females and not by the male. The following table of rearing records show that some females produce female progeny while others produce male progeny.

TABLE 4. *Sex progeny of individual females*

Number	Females	Males	Number	Females	Males
1	135	4	11	0	112
2	0	103	12	43	0
3	0	64	13	0	57
4	30	0	14	2	63
5	8	108	15	0	55
6	64	0	16	75	0
7	76	2	17	46	10
8	90	0	18	69	0
9	0	93	19	53	0
10	111	4	20	0	72

A review of the literature disclosed that Metz (1925) had previously observed this peculiar phenomenon in *Neosciara similans*, and he has shown that this condition occurs in several other species of Neosciara. He showed that the genetic basis responsible for sex of progeny was inherited in a simple Mendelian fashion. The female-producing female breeds as if heterozygous, and the male-producing female breeds as if homozygous recessive, for the gene or gene complex responsible for sex of progeny. Furthermore, he showed that the sex of the individual, as distinguished from sex of progeny, appeared to be dependent on an ordinary XX-XY sex-chromosome mechanism, the sperms containing XY chromosomes, therefore, were sex determining. As a male, when mated to two or more females, might give rise to both sons and daughters, it was assumed that he produced X-bearing and Y-bearing sperms in equal numbers. Metz's experiments indicated that in the female there was an elimination or inactivation of some of the sperms, which led him to conclude that in the female-producing female, the eggs were fertilized only by X-bearing sperms, while in the male-producing female only the Y-bearing sperms functioned, thus leading him to believe that sex was determined by two factors or complexes; the one acting directly and the other indirectly. The sex of the individual fly then depends directly on the type of sperm fertilizing the egg, but the type which would so function depends on the zygotic constitution of the female producing the egg.

CONTROL

Nicotine sulphate (Black Leaf 40), mercuric chloride, mercurous chloride, naphthalene, and calcium cyanide were used in an effort to determine an effective means of destroying Neosciara maggots. Naphthalene and calcium cyanide effectively destroyed the maggots when scattered over the soil in which they were feeding, but these compounds were decidedly harmful to the plants. Mercuric chloride at a concentration of

one ounce to eight gallons of water and mercurous chloride at a concentration of three to five ounces to ten gallons of water proved quite effective when the soil was thoroughly drenched with these concentrations. Nicotine sulphate was not effective in controlling the maggots. Several writers have stated that Neosciara maggots, from soil drenched with nicotine solutions, would not develop functional reproductive organs and that the females, therefore, would die without ovipositing. Data obtained by the writer from experiments conducted with this point in view did not indicate that the reproductive organs were impaired by the application of nicotine solutions, as flies emerging from larvae that had been feeding in soil thoroughly drenched with nicotine sulphate solutions reproduced normally.

Although maggots can be destroyed in the soil by the application of mercuric chloride and mercurous chloride solutions, they can also be easily kept in check, wherever it is practical to do so, by allowing the soil to dry out occasionally. Some of the plats of peas used in these experiments were watered daily, while other plats were watered twice a week or whenever the peas began to show need of water. The peas that were watered daily became heavily infested with Neosciara maggots resulting in serious injury, while those that were not watered so often showed only slight infestation, and apparently the peas were not injured. Several plats of peas were watered from below, thus allowing the surface of the soil to remain dry, with the result that they did not become infested with the maggots. A dry surface is very unattractive to the ovipositing females and even when they do oviposit there the eggs will not hatch as they require contact moisture. Sand also makes an unattractive surface for the ovipositing females, and where valuable plants are being injured by Neosciara maggots they can be protected by covering the surface of the soil with about one-half inch of dry sand.

A small predatory mite was very abundant in greenhouses at Ames, Iowa, and became so numerous that it quite effectually reduced the flies. The mites are predacious on the eggs of Neosciara, and often may be seen clinging to the bodies of the adults.

SUMMARY

Neosciara maggots, when occurring in large numbers, are capable of causing a great deal of damage to plants. They are easily disseminated in soil around plants; therefore, are probably widely distributed.

Although *N. ocellaris* breeds throughout the year in greenhouses, it is more abundant there during the winter and spring; they breed out doors only during the warmer months of the year. The adults frequent moist, shady places, and the eggs will not hatch and the larvae will not develop unless they are in contact with moisture.

At 25° C. and a high relative humidity (nearly 100 per cent), the eggs hatch in four to five days. Under natural conditions, the eggs are laid in clusters consisting of three to 40 eggs; the egg clusters being placed in crevices of the soil or along the underground stems of plants.

The larva is distinguishable from most dipterous larvae by a strongly sclerotized, shining black head. The body is semi-transparent, thus making it possible to see quite distinctly the internal organs.

On decomposing food, the average lengths of the instars are as follows: First 3 days, second 2 days, third 1 day, fourth 5 days; but when feeding on fresh food the larval life is lengthened 4 days. The maggots tend to be omnivorous, feeding on a large number of greenhouse and potted plants as well as on decaying animal tissues. Injury to the roots results in a sudden wilting and death to seedling plants, a result quite similar to that caused by certain seed bed diseases. The larva constructs a silken cocoon in the soil and transforms to an exarate pupa. The pupal stage, at 25° C. is four to five days.

The adults under natural conditions apparently feed exclusively on the ooze from decomposing organic matter; however, captive adults feed readily on a 10 per cent sucrose solution.

Mating occurs soon after the adults emerge, and normally oviposition begins in about two days.

The maggots can be controlled in the soil by drenching the soil with a mercuric chloride solution at a concentration of one ounce to eight gallons of water, and with a mercurous chloride suspension at a concentration of three ounces to ten gallons of water. They can also be kept in check by allowing the soil to dry out occasionally.

Neosciara ocellaris, as well as other species of Neosciara, produce "unisexual" progenies, but there are occasional exceptions.

A small predatory mite was common on the eggs of *N. ocellaris* in greenhouses at Ames, Iowa, and it became so numerous that it quite effectually reduced the population of flies.

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THERMOGENESIS IN HAY-INHABITING FUNGI[1]

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Thermogenesis by certain fungi having been demonstrated by Gilman and Barron (7), Miehe (18) and Norman (19), the way was opened for a more extensive investigation of the phenomenon. The question of how widespread the phenomenon was among other fungi and the relationship of heat liberation to respiration and growth of the fungous thallus were among the problems that were presented by the earlier work. In order to point the investigations to a definite end a substrate (hay) in which heating occurred frequently in nature, was selected; a definite number of organisms (14) commonly found on this substrate were isolated and the relations between heat production, carbon dioxide generation and growth were observed. The results of these observations are reported. Since the literature has been well reviewed in the papers cited above, the interested reader is referred to those authors for the history of microbial thermogenesis.

MATERIALS AND METHODS

ISOLATION OF ORGANISMS

The fungi which were used in these investigations were from two sources; first, isolations made from alfalfa hay which had heated spontaneously to about 60° C. in an experimental storage mow (11), and second, similar isolations obtained from some good quality hay that had been thoroughly wetted with sterile distilled water and incubated at room temperature.

Mow III from which the isolations were made had an initial moisture content of 36.8 per cent, being one of several mows of alfalfa hay stored with various initial moisture contents, for the study of the relation of moisture content to the keeping qualities of the hay. When this mow was opened many yellowish green patches were noted upon the hay; these proved to be composed of fruiting heads of *Aspergillus flavus* Link. Several samples of the hay from various levels to represent the whole were placed immediately in sterile moist-chambers. Later these samples were divided into three portions, which were incubated at room temperature, at 30° C., and at 40° C., respectively.

As conidial heads appeared during the incubation period, they were picked off with a sharp nichrome needle and streaked on hay infusion agar in petri dishes. These streaks were allowed to stand for a few days at room temperature and were examined frequently. The pure cultures were transferred to agar slants. In the case of mixed cultures, spore suspensions in sterile water blanks were prepared and streaks made of the spore suspension; pure colonies were rather easily obtained by this means. Only four species of fungi were represented: *Aspergillus flavus*, *A. fumigatus* Fres., *A. terreus* Thom, and *Rhizopus tritici* Saito.

From the above substrate only the thermoduric forms of fungi were obtained. However, each of these forms proved able not only to endure heat, but also to raise considerably, by its own metabolic activity, the temperature of certain substrates. In addition to the thermoduric forms, many other fungi normally inhabit alfalfa hay. In order to isolate the latter, some good quality hay was thoroughly wetted with sterile, distilled water and incubated at room temperature. As conidia of fungi appeared they were picked off and transferred to agar slants. In addition some of the hay was washed with sterile, distilled water and the washings plated. Many colonies of fungi and bacteria appeared on the plates. The fungi were transferred to agar slants and the bacteria discarded. The following species were obtained: *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. niger* Van Tieghem, *A. clavatus* Desm., *Penicillium oxalicum* Currie and Thom, *P. humicola* Oudemans, *Spicaria divaricata* (Thom) Gilman and Abbot, *Mucor abundans* Povah, *Rhizopus tritici*, *R. nigricans* Ehrenberg, *Cunninghamella elegans* Lendner, *Hormodendron nigrescens* Paine, and *Alternaria humicola* Oudemans.

This group of fungi represents the more common inhabitants that may be found on hay growing under the conditions existing at the time the isolations were made. It is not to be considered a complete flora of that substrate.

In subsequent experiments several of these forms were found to release heat in considerable quantities although unable to survive the higher temperatures.

PREPARATION OF SUBSTRATE

For identification and for the study of growth rates, Czapek's medium, prepared in the usual manner (24), was used in all cases.

Alfalfa hay for the thermogenesis experiments was obtained from the Iowa State College dairy farm. This hay was selected because it was of good quality, and it had been chopped by machinery into short, rather uniform lengths convenient for manipulation. A quantity of approximately sixty grams of the hay was placed in a number of glass tubes, 18″ x 1¼″, which were plugged at each end with cotton, and sterilized at 15 pounds pressure for one hour on each of four consecutive days. This treatment, though severe, was found to be essential to assure complete sterilization. The moisture content was then determined. This determination was necessary in order that the proper adjustment could be made by the addition of sterile water at the time of inoculation.

PREPARATION OF THE FLASKS

Ordinary commercial thermos flasks, 40 cc., capacity, were used in all experiments. These flasks were first thoroughly washed in boiling water

and then kept in a 1-500 solution of mercuric chloride for several days. Just before using, they were rinsed several times, first with scalding water from the high pressure steam boilers, and finally with several changes of cool sterile, distilled water. The latter helped to cool the flasks to room temperature. The flasks were closed with rubber stoppers which had been treated with 50 per cent alcohol. It was later found by Gaskill (6) that the flasks could be plugged with cotton and successfully sterilized in the autoclave.

<center>PACKING THE FLASKS</center>

To pack the flasks the plug was first removed from one end of the tube of hay, inverted over the mouth of a sterile flask; the hay was pushed ahead of the upper plug into the flask by means of a glass rod. The flask was immediately closed with a rubber stopper which had been treated with 50 per cent alcohol. The operation was carried on in the inoculating chamber and the usual precautions taken to prevent contamination.

<center>INOCULUM AND INOCULATION</center>

In the inoculation of the substrates, there were two problems to be considered: the even distribution of the spores and the proper adjustment of the moisture content. Large cultures of the desired organism were grown on agar slants in sixteen-ounce flat bottles. As soon as sufficient quantity of spores was produced, the slant was washed with enough sterile, distilled water to bring the contents of the flask to the desired moisture content. The spore suspension was poured into the hay and the flask was closed with either a sterile rubber stopper or a cotton plug, the stopper carrying a sterile thermometer. The inoculated flasks were then rolled for about fifteen minutes to even the distribution of the moisture and of the spores, after which they were allowed to lie on the side for an hour during which they were rolled at frequent intervals.

For control there was used a flask loaded with sterile hay, brought to the same moisture content as the inoculated flasks. In a few cases a dry control was also used, but as it made no contribution of useful information, it was dropped from subsequent experiments.

<center>METHODS OF OBSERVATION</center>

<center>*Method of Determining Growth Rates*</center>

Growth rates were determined on petri dishes containing 10 cc. of Czapek's medium streaked with three drops of a spore suspension of the organism to be observed. A sufficient number of plates was inoculated to allow one or more to be incubated at each interval of five degrees from 0° C. to 50° C.

As germination occurred, the developing hyphae were measured frequently with an eyepiece micrometer until the spore could no longer be seen. As a rule twenty developing hyphae were selected at random over a small area, and measured, and calculation made of their average length. To give some index of comparison, the number of microns of growth was divided by the number of hours and the quotient recorded as the growth index.

This program was carried on in convenient series until all the organisms had been grown at each temperature interval. Although it was necessary to divide the work into small sections to facilitate handling, the conditions of the experiment were kept as nearly constant as possible.

Determination of Thermogenesis

During the study of thermogenesis two objectives were sought: first, a measurement of the rise in temperature, and second, the relation of this rise to respiration. Therefore, two series of observations were made; one to determine temperature alone and another to obtain temperature readings simultaneously with the measurement of carbon dioxide production.

In the first series the flasks were closed with cotton plugs and allowed to stand on the laboratory tables, subject to the temperature changes of the room. Readings were made by means of thermometers, usually at 9:00 a. m. Record was made of each flask, of the control, and of the room temperature[3].

In the second series the flasks were so arranged that temperature readings could accompany the determination of carbon dioxide evolution. For this purpose a thermocouple was passed through the rubber stopper into the mass of heating material and the temperature read on the dial of a potentiometer graduated directly in degrees Fahrenheit. These readings were immediately converted into degrees Centigrade and recorded. At each reading the accuracy of the potentiometer was checked against a calibrated thermometer in warm water and the instrument adjusted as required. To facilitate handling a number of flasks, a rotary switch was placed between the flasks and the potentiometer.

Determination of Carbon Dioxide Evolution

To determine the rate of carbon dioxide evolution, special absorption towers (Fig. 1), a modification of those described by Emerson (5), were built. Immediately following the first daily temperature reading, the vitiated air was drawn from the flasks and passed through a n/10 solution of barium hydroxide. Aspiration was continued until the entire air of each flask had been displaced, as nearly as possible, by carbon dioxide-free air. After allowing sufficient time for the precipitate to settle out, the collection tubes were detached and the contents rapidly filtered through a tared filter paper. After drying, the precipitate was weighed, and the carbon dioxide solution in milligrams per hour was calculated. This method can be relative only, but should furnish an index of the relation between carbon dioxide and the rate of heat production.

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[3]Observations were also made on several organisms with the flasks placed in the constant temperature bath.

The purpose of the experiments carried on in the water bath was to determine the effect of the fluctuations of room temperature on the progress of the heat curve and on the maxima. The observed differences in temperature between a series of flasks held in the constant-temperature bath and a series held at room temperature were no greater than the differences between individual members of either of the series. For the present experiment, the water-bath was considered an unnecessary refinement.
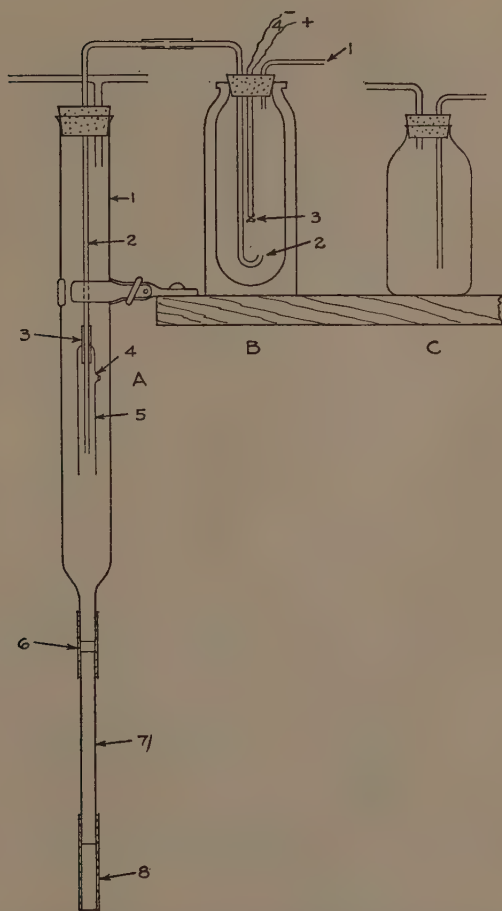
Fig. 1. A. The CO_2 absorption apparatus. 1. Shell filled with $Ba(OH)_2$. 2. Intake tube from flask. 3. Rubber to hold 5 in place. 4. Outlet from absorption shell. 5. Absorption shell made out of test tube. 6. Rubber connection with detachable tube. This connection may be closed by means of a pinch cock. 7. Detachable tube for collecting $BaCO_3$. 8. Rubber tube to facilitate refilling. 9. Tube connecting units in series, and the series with the suction pump.

B.   Thermos flask with attachments. 1. Intake for CO_2 free air. 2. Outlet for vitiated air connected to A2. A. Copper-constantin junction. 4. Insulated wires to switch-board.

C. Trap containing strong KOH.

EXPERIMENTAL DATA

PRESENTATION OF RESULTS

In order to carry out a logical presentation of the results, the data on growth have been separated from those on the temperature-carbon dioxide relations of the organisms concerned. These two sub-groups were further divided for convenience so as to bring the data collected from related organisms together. Three natural groups occur: the species of Aspergillus, the Mucorales, and the Hyphales, containing two species of Penicillium and one each of Spicaria, Hormodendron, and Alternaria.

The data presented in all cases are summaries of the readings made on several individual cultures or flasks. No carbon dioxide readings are recorded for any of the controls since the amount of carbon-dioxide given off by the control flasks was so small that it was not measurable by the means used in these experiments.

GROWTH INDEX

As explained under materials and methods, the growth index for each organism was obtained by measuring the actual growth increment in microns for a given time and dividing the number of microns obtained by the number of hours required to make the increase.

When the data on the growth indices of the five species of Aspergillus (Table 1) are examined, it is evident that except at the optimum temperature or above, the most active rate of growth occurred in the 25-84 hour period. In the case of *Aspergillus flavus* and *A. niger* the growth at the optimum 30° C. was greatest during the 9-24 hour period. With *A. terreus* and *A. clavatus* the growth rate during the second and third period was the same at the optimum temperature of 35° C. *Aspergillus fumigatus* had the highest optimum at 40° C. Its highest index, 64, occurred in the 25-48 hour period. Above the optimum, the growth rate of the third period, 25-48 hours, was frequently less than that of the second. A similar condition was found with the members of the Mucorales studied. (Table 2.) In *Mucor abundans* the optimum was apparently at 25° C., although high indices were found at 30, 35, and 40° C. Except at the last temperature the rate was greatest during the third period, reaching its maximum at 130 at 25° C. The optimum for *Rhizopus tritici* was also 25° C. during the third period with an index of 350. *Rhizopus nigricans* showed a lower optimum between 20 and 25° C., but the growth during the period 25-48 hours was so rapid that measurements were impossible since the hyphae had completely covered the surface of the culture dish.

In the case of the third group of fungi the greatest growth occurred in the third period of time except at 35° C. with *Penicillium oxalicum*, at 10, 25, and 30° C. with *Alternaria humicola* (Table 3), and at 10 and 15° C. with *Spicaria divaricata*. The optimum temperatures for the five organisms under the conditions used were: *Penicillium oxalicum*, 25° C.; *P. humicola*, 20° C.; *Spicaria divaricata*, 30° C.; *Hormodendron nigrescens*, 15° C.; and *Alternaria humicola*, 25° C. The indices follow the general trend of the previous groups; that is, when the temperature was such that there was markedly stimulated growth in the second period, there was usually a rapid fall in growth rate in the succeeding period.

TABLE 1. Growth indices for the species of Aspergillus as determined by μ/hr.

Degrees C.	Aspergillus flavus			Aspergillus terreus			Aspergillus niger			Aspergillus fumigatus			Aspergillus clavatus		
	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0		3	0	0	0	0	3	6	0	0	0	0	0	3
15	0	0	1	0	0	0	0	3	7	0	0	1	0	5	78
20	0	10	66	0	0	0	0	9	121	0	0	0	0	2	39
25	0	9	297	0	2	46	0	12	114	0	3	40	3	6	50
30	17	490	333	0	2	46	16	286	333	0	2	40	15	24	80
35	0	88	88	0	83	83	10	200	321	0	40	40	0	40	40
40	3.6	66	41	0	47	47	6	248	144	0	58	64	0	4	12
45	0	45		0	24	17	0	4	0	0	58	0	0	3	0

TABLE 2. *Growth indices for the species of Mucorales as determined by* $\mu/hr.$

Degrees C.	Mucor abundans			Rhizopus tritici			Rhizopus nigricans			Cunninghamella elegans		
	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.
10	0	1	13	0	2	16	0	5	6	0	0	30
15	0	0	6	0	12	70	0	14	56	0	51	103
20	0	22	114	0	.....	187	9	38	*	0	85	175
25	6	50	130	5	200	350	7	60	*	8	200	.....
30	0	97	127	0	60	100	0	0	0	5	416	.....
35	0	80	110	8	60	100	.....	.....	.....	7	400	.....
40	40	150	110	12	85	100	.....	.....	.....	0	555	.....
45	0	0	0	0	80	0	.....	.....	.....	.....	.....	.....

TABLE 3. *Growth indices for the species of Penicillium, Spicaria, Hormodendron, and Alternaria as determined by μ/hr.*

Degrees C.	Penicillium oxalicum			Penicillium humicola			Spicaria divaricata			Hormodendron nigrescens			Alternaria humicola		
	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-24 hrs.	25-48 hrs.	1-8 hrs.	9-25 hrs.	25-48 hrs.	1-8 hrs.	8-24 hrs.	25-48 hrs.
10	0	2	7	0	0	2	0	7	4	0	10	20	9	82	23
15	0	3	11	0	4	36	0	7	4	0	20	26	0	80	100
20	2	5	11	0	27	42	0	18	35	10	10	11	10	72	82
25	0	30	45	2	15	25	0	15	30	0	0	0	60	200	100
30	0	24	42	0	3	5	0	17	35	⋮	⋮	⋮	53	287	63
35	1	70	38	0	2	2	0	10	30	⋮	⋮	⋮	4	35	70
40	0	0	0	0	0	0	0	10	76	⋮	⋮	⋮	2	5	30
45	0	0	0	0	0	0	0	0	0				0	0	0

In general, each form studied increased its growth activity as temperature increased, until the optimum was reached, after which a sharp decrease in growth occurred. However, there were some apparent exceptions; for example, in *Penicillium oxalicum*. In the study of this form it was found that at 10° C. the growth index was 18, at 15° C. it was 71 and at 20° C., a very favorable growth temperature, the growth index was only 30. A closer examination of the experimental plates showed that at 15° C. only 50 per cent of the spores had germinated, but at 20° C. germination was 100 per cent. Increased germination resulted in crowding and inhibition of the growth of the individual hyphae. Microscopic measurement of the individual hyphae gave a greater growth index at 15° C. than at 20° C., but there was no doubt that if the quantity of mycelium per spore seeded could have been accurately determined, it would have been much greater at 20° C. It was purely a case where the acceleration of increased temperature was neutralized by the inhibiting effects of crowding. Several forms, especially those which tolerated or preferred the higher temperatures, were able to grow over a wide temperature range. A good example was *Aspergillus fumigatus,* which had its optimum above 40° C., which was able to make growth at 50° C., and, if allowed sufficient time, could grow well at 10° C. The growth activity of *Cunninghamella elegans* was very interesting. At all the temperature intervals from 10° C. to 40° C. rapid growth occurred. From 25° C. to 40° C. the petri dishes were completely filled with mycelium in 48 hours. Another form, *Spicaria divaricata,* grew over a wide range of temperature, though the amount of mycelium produced at any time was very small. Two other forms, *Hormodendron nigrescens* and *Penicillium humicola,* preferred temperatures below 20° C. In table 4 is shown the optimum temperature for each form studied as determined by the growth index method.

TABLE 4. *Growth optima of organisms studied*

15°-20°C.	25°C.	30°-35°C.	40°C.
Hormodendron nigrescens	Rhizopus nigricans	Aspergillus flavus	Aspergillus fumigatus
Penicillium humicola	Alternaria humicola	A. terreus	
	Penicillium oxalicum	A. clavatus	
		A. niger	
		Rhizopus tritici	
		Mucor abundans	
		Spicaria divaricata	
		Cunninghamella elegans	

THERMOGENESIS

In all the experiments in thermogenesis alfalfa hay was used as the substrate. The severe treatment of the hay in sterilization may be open to some criticism because of chemical changes brought about during the process. All hay used in the experiment was treated in exactly the same manner; therefore, benefits or handicaps were the same for each organism studied and would not seriously affect the results. It was obvious that no living tissue of the alfalfa survived.

From the results which have been obtained it was shown that certain fungi were able to raise the temperature of alfalfa hay after all factors, other than those involved in the vital processes of the fungi themselves, had been eliminated. All the forms studied were not equal in their power to release heat; in fact, the thermogenic ability of some was almost negligible under the conditions of the experiment. These non-thermogenic species were usually either very slow in growing or they preferred lower temperatures for their maximum activities. It is easy to believe that such forms produced heat but that it was liberated no faster than it was dissipated. Figure 13 shows graphically that very little heat was produced by *Spicaria divaricata*. While this form grew over a wide temperature range, it produced only a small amount of mycelium. Another form, *Hormodendron nigrescens*, as shown in table 3, had an optimum growth temperature of 15° C. The temperature of the flask inoculated with *H. nigrescens* was at no time more than five degrees above the control. (Fig. 14.) It is possible that rising temperatures inhibited growth, and that inhibition is reflected in the release of heat.

Aspergillus flavus[4] was the most active heat producer on hay. When placed on any suitable medium it germinated quickly and grew very rapidly. This characteristic was reflected in the temperature produced. As may be seen in figure 2, the temperature of a flask inoculated with *A. flavus* started to rise in a very short time, and continued until the maximum of 40° C. was reached on the third to fifth day. After the maximum was reached there was a rapid decline to a few degrees above the inoculated control flask. The new level was held for several days if the flasks were not opened. Two other species of Aspergillus found to be definitely thermogenic were *A. terreus* (Fig. 3) and *A. niger* (Fig. 4). Both forms raised the temperature of their substrate to about 40° C. or 14° C. above the control. In the present experiment, *A. fumigatus* (Fig. 5) released comparatively little heat. This was disappointing in view of the findings of several other investigators, namely, Miehe (18), James, Rettger and Thom (15), Norman (19) and Gilman and Barron (7). *Aspergillus fumigatus* was found by these workers to be very active on several types of substrate. For example, the data of Gilman and Barron show a maximum of 53° C. when grown on oats and of 31.2° C. on wheat. These data indicate that the type of substrate affects the amount of heat released by fungi. Hay, perhaps, was not a good substrate for *A. fumigatus*, or it may have been that the initial temperature was too low for the maximum activity of the fungus.

It was very interesting to note that the two species of Rhizopus and one species of Mucor were thermogenic[5]. *Rhizopus tritici* (Fig. 9) and

[4]Table 5.
[5]Table 6.

Fig. 2. *Aspergillus flavus.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
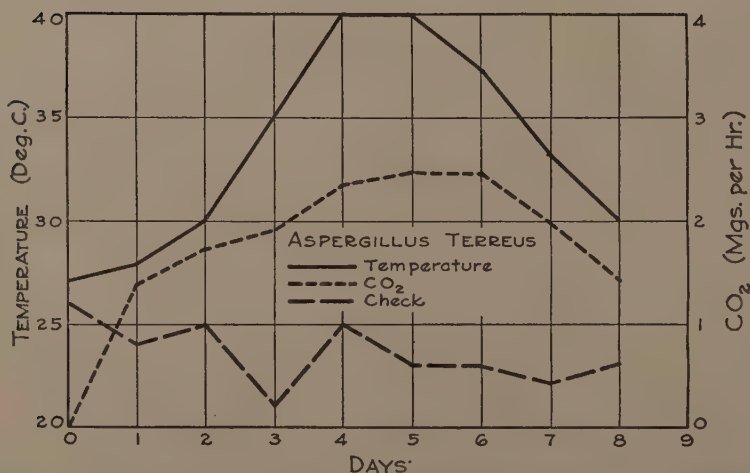
Fig. 3. *Aspergillus terreus.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

TABLE 5. *Temperature and carbon dioxide production in 60-gram samples of alfalfa hay by species of Aspergillus*

Days	Aspergillus flavus		Aspergillus terreus		Aspergillus niger		Aspergillus fumigatus		Aspergillus clavatus		Check	
	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.
0	25.0	1.0	27.0	1.4	25.0	0.4	21.0	2.3	23.0	1.95	24.4	0
1	30.5	1.8	27.8	1.7	26.0	1.95	22.3	1.7	24.4	1.36	24.2	0
2	33.0	2.6	30.0	1.85	28.0	2.1	23.7	1.9	24.0	3.0	23.8	0
3	33.0	2.5	35.0	2.4	31.0	2.0	25.6	1.6	23.7	3.9	23.0	0
4	38.5	2.6	40.1	2.45	34.9	2.25	24.5	1.1	24.4	1.25	24.2	0
5	40.0	2.6	40.0	2.5	34.0	2.9	24.7	1.1	25.2	1.3	23.8	0
6	37.0	3.1	37.0	2.0	29.0	2.25	25.3	2.7	26.6	1.3	24.2	0
7	36.5	2.8	33.0	1.6	27.0	2.25	26.9	2.1	24.0	1.3	23.4	0
8	33.0	2.1	30.0		26.5		26.0	2.0	....	....	23.4	0

Readings taken at 9 a. m. daily. Each reading, including control, is composite of several observations. No carbon dioxide was found in controls.

TABLE 6. *Temperature and carbon dioxide production in 60-gram samples of hay by species of the Mucorales*

Days	Mucor abundans		Rhizopus nigricans		Rhizopus tritici		Cunninghamella elegans		Check	
	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.	Temp. degrees C.	CO_2 mg. per hr.
0	22.4		23.0		25.0		26.0		24.5	0
1	24.1	1.2	23.0	1.8	21.0	1.6	27.0	2.1	23.0	0
2	26.0	1.5	23.0	1.6	39.5	2.1	29.5	2.0	24.0	0
3	27.2	2.0	25.0	2.1	42.0	2.2	36.5	2.7	23.2	0
4	28.2	2.0	28.0	2.3	34.0	2.3	30.5	3.1	24.0	0
5	24.5	2.2	30.5	2.2	30.5	1.6	29.5	2.5	23.0	0
6	24.0	2.2	32.5	2.1	30.0	1.0	29.5	3.0	22.0	0
7	23.5	2.0	31.5	2.0	28.5	1.1	29.0	2.5	23.0	0
8	24.0	1.2	30.0	1.5	27.0	1.0	25.0	2.2	24.0	0

TABLE 7. *Temperature and carbon dioxide production in 60-gram samples of hay by species of Penicillium, Hormodendron and Alternaria*

Days	Penicillium oxalicum Temp. degrees C.	Penicillium oxalicum CO₂ mg. per hr.	Penicillium humicola Temp. degrees C.	Penicillium humicola CO₂ mg. per hr.	Spicaria divaricata Temp. degrees C.	Spicaria divaricata CO₂ mg. per hr.	Hormodendron nigrescens Temp. degrees C.	Hormodendron nigrescens CO₂ mg. per hr.	Alternaria humicola Temp. degrees C.	Alternaria humicola CO₂ mg. per hr.	Check Temp. degrees C.	Check CO₂ mg. per hr.
0	24.0		24.0		28.5		21.6		23.0		26.0	0
1	27.5	0.4	25.5	0.8	28.5	0.3	24.3	0.7	23.0	0.6	24.0	0
2	29.5	0.6	25.0	0.7	28.0	0.5	25.1	3.1	26.0	0.9	24.4	0
3	32.0	1.1	24.5	1.3	27.5	0.5	25.1	2.0	26.0	3.9	24.4	0
4	34.5	1.0	22.0	1.4	26.5	0.3	22.8	1.4	25.5	0.9	24.0	0
5	32.5	1.0	26.0	0.7	27.0	0.3	22.8	1.0	25.5	4.6	23.0	0
6	31.0	1.2	23.0	1.3	27.0	0.1	25.4	1.3	25.0	3.1	23.0	0
7	30.0	1.1	25.0	1.4	26.5	0.1	23.5	1.6	25.0	4.4	22.0	0
8	28.5	1.0	24.0	1.0	27.5	0.1	26.0	1.0	27.0	3.6	25.0	0

Each column, including check, is composite of readings of several observations. No carbon dioxide was found in the check.

R. nigricans (Fig. 10) raised the temperature of their substrates to 42.9° C. and 32° C., respectively. *Mucor abundans* (Fig. 7) reached only 28.5° C., but this point was 6.5° C. above the check flask. Miehe (18) reported 38° C. for *R. nigricans* and over 50° C. for *Mucor corymbifer*.

Another of the Phycomycetes, *Cunninghamella elegans*, was found to release exceptional quantities of heat. The series of experiments from which the data graphically recorded in figure 10 were obtained, showed a maximum temperature of 37° C. on the third day, but in another observation over 40° C. was obtained the second day. This form grew rapidly over a wide range of temperature and in addition it produced a large amount of mycelium.

Of the species of Penicillium studied[6] *P. oxalicum* (Fig. 11) was thermogenic while *P. humicola* (Fig. 12) was not. Studies on growth showed that *P. humicola* preferred a lower temperature for growth than did *P. oxalicum*. This difference in growth habit was reflected in the rapidity in which temperature was raised.

If the amount of heat released by fungi were studied on a quantitative basis by a series of experiments conducted with a calorimeter, the relative efficiency of each organism could, no doubt, be established.

A summarized list of the species studied for heat production with the maximum temperature attained, the difference between the maximum of the inoculated flask and the check, and the day on which the maximum was reached, for each form is given in table 8.

TABLE 8. *Maximum temperatures in degrees Centigrade developed by organisms grown on sterile alfalfa hay. Forty per cent moisture content*

Organisms	Maximum Temp.	Temp. of control	Maximum temp. above control	Day
Aspergillus flavus	44.4	25.0	19.4	3
A. terreus	41.0	27.0	14.0	4
A. niger	39.4	26.0	13.4	6
A. fumigatus	27.0	24.0	3.0	7
A. clavatus	26.6	24.0	2.6	6
Penicillium oxalicum	34.0	24.0	10.0	4
P. humicola	26.0	24.0	2.0	5
Spicaria divaricata	27.0	26.0	1.0	4
Mucor abundans	28.5	22.0	6.5	5
Rhizopus tritici	42.9	26.0	16.9	3
R. nigricans	37.0	23.0	14.0	6
Cunninghamella elegans	40.0	26.0	14.0	2
Hormodendron nigrescens	25.5	20.5	5.0	4
Alternaria humicola	27.0	24.0	3.0	5

Thermogenesis and Carbon Dioxide Production

In an attempt to correlate thermogenesis with respiration in the fungi studied, the data showed in some cases a rather close similarity in the progress of the curve for carbon dioxide production to the curve of temperatures.
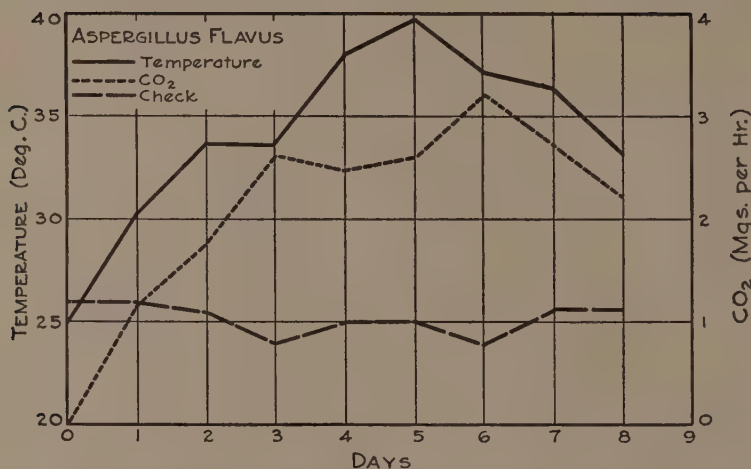
[6]Table 7.
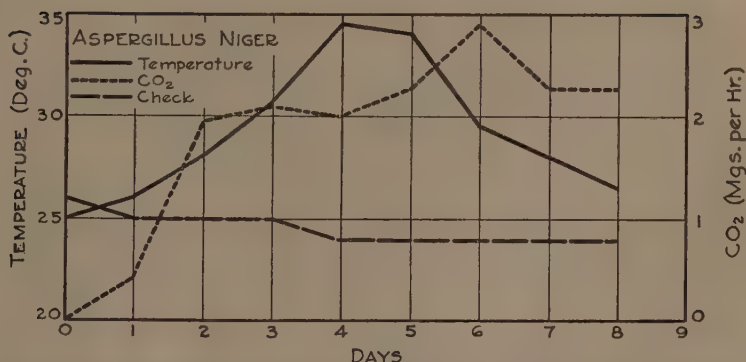
Fig. 4. *Aspergillus niger*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
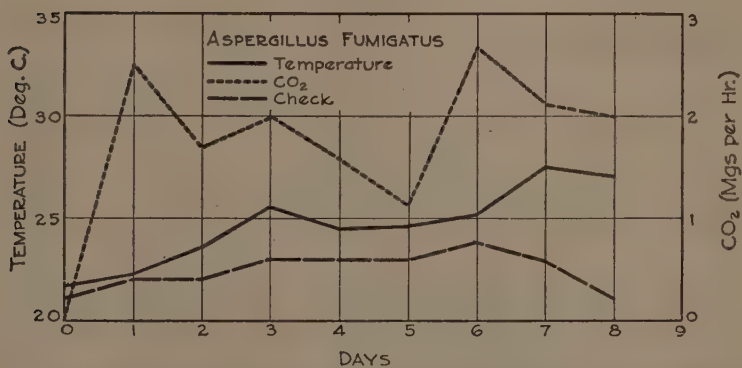
Fig. 5. *Aspergillus fumigatus*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

With *Aspergillus flavus, A. terreus, Mucor abundans, Rhizopus tritici,* and *Penicillium oxalicum,* the curves approximate each other rather well. In other cases, *Aspergillus niger, A. fumigatus, A. clavatus, Rhizopus nigricans, Cunninghamella elegans, Penicillium humicola, Hormodendron nigrescens* and *Alternaria humicola,* the carbon dioxide curve was indicative of much greater activity than was shown by the temperature recorded, and there was no apparent correlation of the two phenomena. With *Spicaria divaricata* the trends of the two curves were opposed. In no case could comparison of activity, unit of carbon dioxide for unit of heat, be made of different species of organisms even within closely related groups. An example of extreme divergence of the behavior of organisms is well illustrated by comparing the data for *Aspergillus flavus* (Table 4) with those of *Alternaria humicola* (Table 6). Here it is found
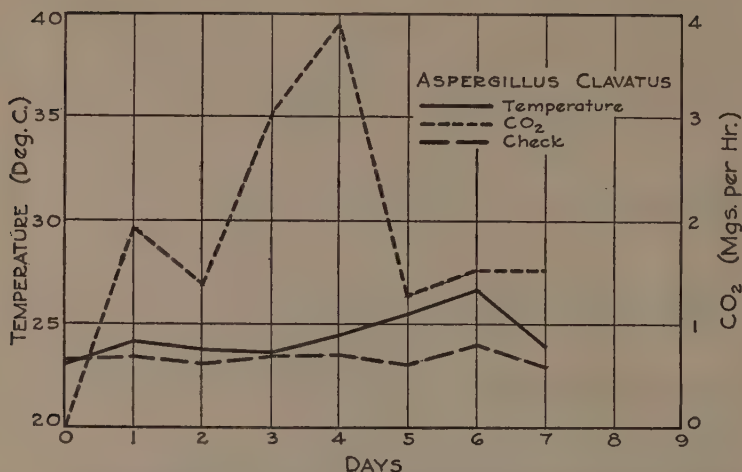
Fig. 6. *Aspergillus clavatus.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
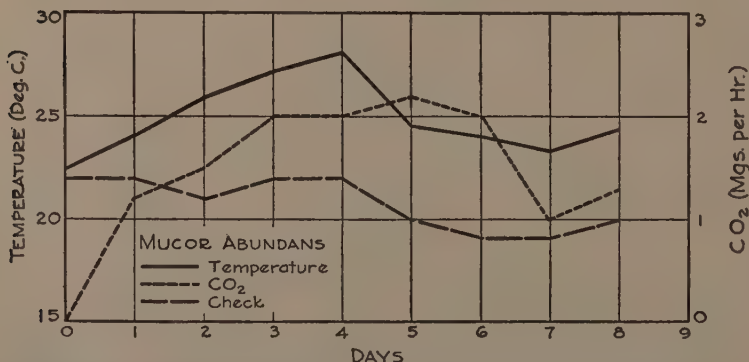
Fig. 7. *Mucor abundans.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

that *Alternaria humicola,* although it produced only enough heat to raise the substrate 3° C. above the check flask, produced a much greater quantity of carbon dioxide than was produced by *Aspergillus flavus,* which raised the temperature of its substrate almost 20° C. above the check flask. In figure 14 the curve for carbon dioxide production for *Hormodendron nigrescens* shows a very rapid rise to the second day followed by a rapid decline to the fifth day. From the evidence of these observations vital activity was more accurately measured by the amount of carbon dioxide produced than by the amount of heat released. Norman (19) obtained a closer relationship between carbon dioxide production and
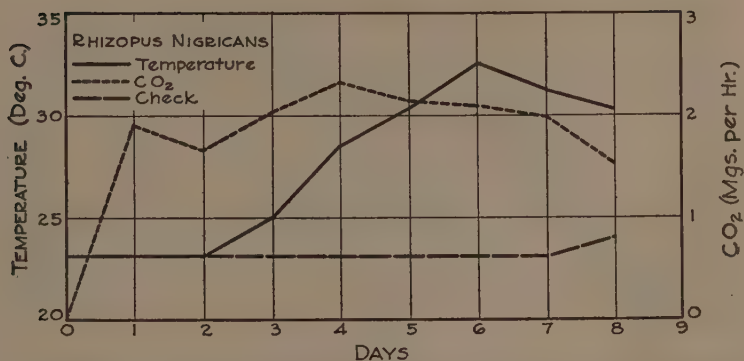
Fig. 8. *Rhizopus nigricans.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
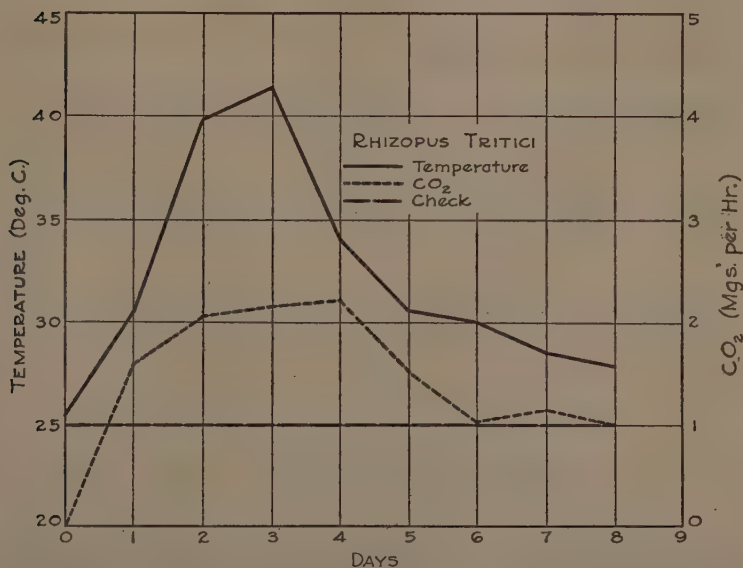
Fig. 9. *Rhizopus tritici.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

heating, both with a mixed micro-flora and with a pure culture of Trichoderma sp. The factors influencing the relation between carbon dioxide and thermogenesis in the investigations here reported were not sufficiently controlled to allow of explanation of the discrepancy found.

When a substrate like hay was used, it was almost impossible to keep conditions for respiration constant, especially in closed vessels. Even with constant aeration, there was no guarantee against local matting of the
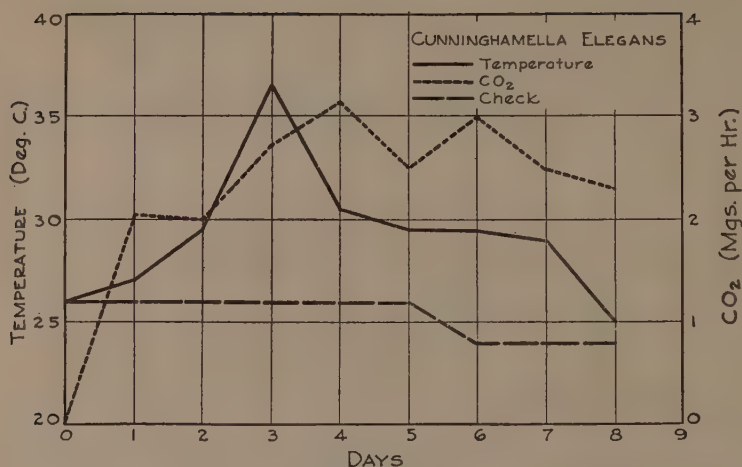
Fig. 10. *Cunninghamella elegans.* Relation of temperature to carbon dioxide produc-
tion in sixty-gram samples of hay.
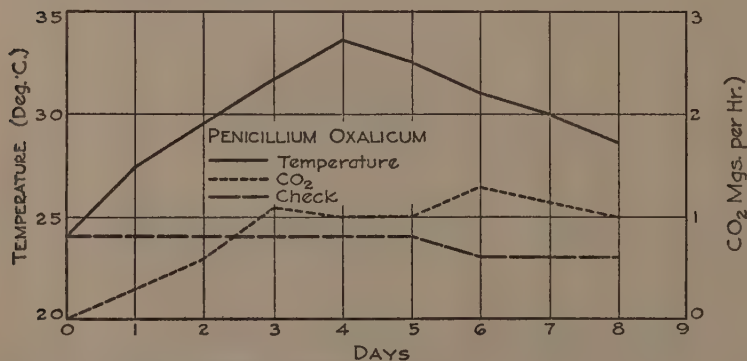
Fig. 11. *Penicillium oxalicum.* Relation of temperature to carbon dioxide production
in sixty-gram samples of hay.

mycelium or caking of the substrate. If possible to section the mass all
gradations from aerobic to anaerobic conditions would be found. Under
anaerobic conditions degradation of carbohydrates would occur with the
release of much smaller amounts of heat than under aerobic conditions.

Thermogenesis and Growth

Those forms of fungi found to be actively thermogenic were, in gen-
eral, capable of active growth and produced large amounts of mycelium.
When the growth indices were compared with the temperature produc-
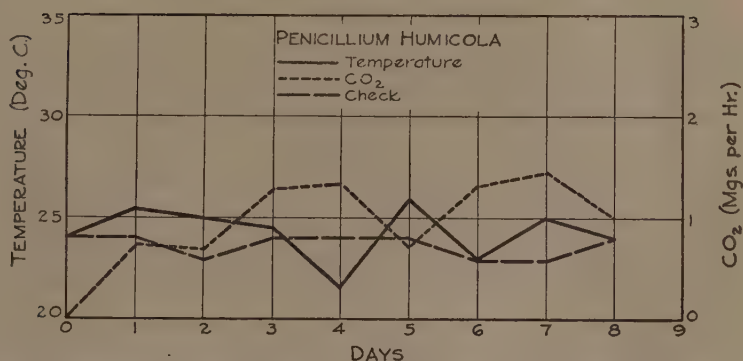
Fig. 12. *Penicillium humicola*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
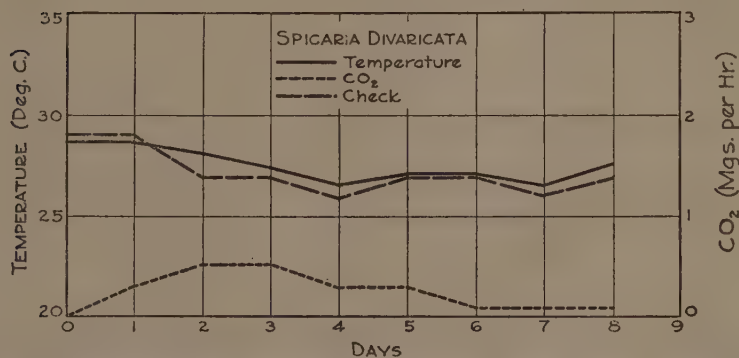
Fig. 13. *Spicaria divaricata*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

tion it was found that the period of rapid accumulation of heat corresponded very closely to the period of rapid germination and growth on artificial media. This was well illustrated in the case of *Cunninghamella elegans*, in which the growth index for the 1 to 8 hour period was 5 and for the 9 to 24 hour period rose to 416 and continued too rapidly for measurement. Figure 10 shows a very rapid rise in temperature from 25° C. to 40° C. in 48 hours. The data indicate that thermogenesis is subordinate to the vital processes involved in growth, and heat results when more energy is released by the organism than is required for its growth.
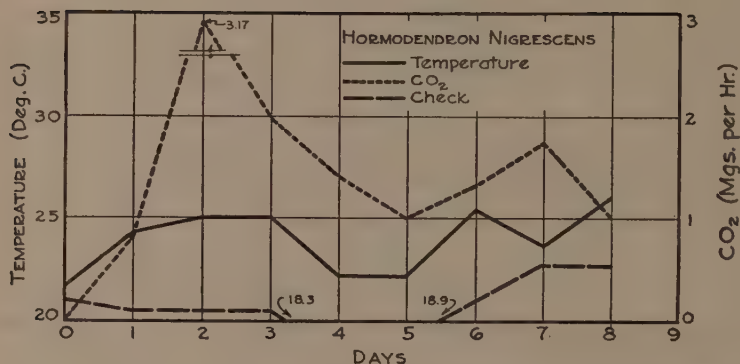
Fig. 14. *Hormodendron nigrescens.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
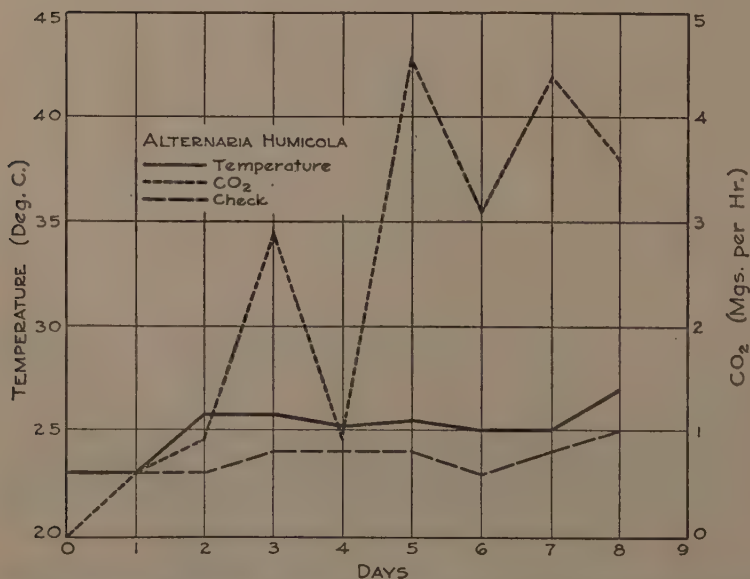
Fig. 15. *Alternaria humicola.* Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

SUMMARY

Fourteen of the common hay-inhabiting fungi were isolated and studied.

The growth response of each form to temperature was obtained on artificial media by measuring the growth increment of the hyphae and dividing by the number of hours required for the increase. This gave a growth index.

The growth optima obtained were: *Hormodendron nigrescens* and *Penicillium humicola,* 15° C. to 20° C.; *Rhizopus nigricans, Penicillium oxalicum* and *Alternaria humicola,* 25° C.; *Aspergillus flavus, A. terreus, A. clavatus, A. niger, Rhizopus tritici, Mucor abundans, Spicaria divaricata* and *Cunninghamella elegans,* 30° C. to 35° C.; *Aspergillus fumigatus,* 40° C.

Each organism was inoculated in pure culture on sterile alfalfa hay in thermos flasks, the substrate brought to 40 per cent moisture content and thermogenesis and carbon dioxide evolution measured.

Each form was able to raise the temperature of its substrate to some degree. *Aspergillus flavus, A. terreus, A. niger, Penicillium oxalicum, Rhizopus tritici, R. nigricans,* and *Cunninghamella elegans* proved to be decidedly thermogenic. *Mucor abundans* and *Hormodendron nigrescens* were thermogenic to a lesser degree.

Under the conditions of the experiments, *Aspergillus fumigatus, A. clavatus, Penicillium humicola, Spicaria divaricata* and *Alternaria humicola* developed little or no heat.

Though the curve for carbon dioxide evolution was in many cases parallel to that for heating, carbon dioxide production could not be used as a measure of thermogenesis.

Periods of rapid accumulation of heat in the inoculated flasks corresponded to periods of active germination and growth of the same organisms on artificial media.

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SHORTENING THE REST PERIOD OF THE TUBERS OF THE JERUSALEM ARTICHOKE, HELIANTHUS TUBEROSUS L.[1]

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Considerable interest has developed in the Jerusalem artichoke, *Helianthus Tuberosus* L., as a crop plant since the tubers of the plant have been found to be a source of production for levulose. Although the plant is native to North America it has never been cultivated here to the extent that it has in Europe, where the tubers are used for human consumption as well as stock feed. If it is to be planted extensively, a number of problems with regard to storage, propagation and seed must be solved. The tubers have a definite rest period after digging before growth will start. Is the length of the rest period influenced by temperature? Can the dormant period be shortened by the use of chemicals? Does the maturity of the tuber affect the rest period and what effect does the maturity of the tuber have on germination? Some experiments are reported in this paper which attempt to answer the foregoing questions.

REVIEW OF LITERATURE

The literature on the rest period of the Jerusalem artichoke tuber is limited, although many reports have been made on the dormancy of potatoes, gladiolus, woody plants, seeds and bulbs. Boswell (2) used 145 varieties or strains of this plant to determine the range in the length of the rest period of the tubers. The time required for 50 per cent of the seed pieces to sprout ranged from 54 to 200 days for the various lots.

Since the Jerusalem artichoke plant produces a tuber, it would be expected that the reactions of the dormant tuber to treatment with chemicals would be similar to those of the tuber of the common potato, *Solanum tuberosum*. Denny (4, 5) found that the vapors of ethylene chlorohydrin were effective in causing sprouting of dormant potatoes. Thiourea also caused prompt sprouting and the development of more than one sprout per eye. A number of workers have employed chemicals of various kinds to break or shorten the rest period of potato tubers. Ethyl bromide was used by McCallum (15). Rosa (16, 17) reported favorable results with nitrate of soda and ethylene gas, although nitrate of soda was not always effective. Loomis (12) recommended storage temperature of approximately 30° C. for shortening the dormant period of potato tubers. Loomis and Evans (14) suggested that vegetative organs containing stored starch will have the rest period shortened by ethylene, ethylene chlorohydrin, ether and similar compounds, while parts of plants such as bulbs containing little starch will not show the same response.

Denny and Stanton (10, 11) found ethylene dichlorid and ethylene chlorohydrin effective in breaking dormancy in several species of woody plants and discovered that dormancy is not systemic but localized in the buds only. Denny (6) treated freshly harvested gladiolus corms of several varieties with ethylene chlorohydrin but the results were variable, depending upon the stage of dormancy and the variety.

Traub et al (18) stored artichoke tubers successfully at 0 to 2° C. (32-35° F.) and a relative humidity of 89-92 per cent. Above 4.4° C. (40° F.) the tubers were found to be more susceptible to storage diseases and the tubers lost moisture rapidly and shriveled.

EXPERIMENTAL

In the fall of 1932 an experiment was under way to determine the effect of harvesting date on the yield of artichoke tubers. Ten hills were dug at weekly intervals, starting Sept. 17 and continuing until Oct. 31, when all the crop was harvested. It was found at that time that the hills produced about eight times as many tubers by weight when harvested on Oct. 31 as when harvested on Sept. 17. Tests made on the levulose content of the early harvested tubers showed them to yield less levulose than tubers harvested a month later. There immediately arose the question of the value of early harvested tubers for seed purposes. Will early harvested tubers or immature tubers germinate satsisfactorily if used for propagation of the following crop? Tubers which had been dug at weekly intervals were stored in a concrete underground storage and plantings made weekly from each lot. Pieces of tubers containing at least one good eye and weighing from one to one and one-half ounces were planted in rich compost soil. Each of 20 pieces from each lot was planted in four-inch pots and placed in a greenhouse where the temperature ranged from 15 to 18° C. The variety used for this study was the Mammoth French White (Sibley strain). Of all the varieties grown on the station grounds this seems to be the best adapted as measured by yields of tubers.

The initial temperature of the storage room where the tubers were stored was about 70° C. There was a gradual decrease in temperature from Oct. 1 to Nov. 1, at which time the storage reached a temperature of about 2° C. Although this was a storage cellar without artificial refrigeration the temperature could be held remarkably constant, with fluctuations averaging about two degrees after Nov. 1.

Table 1 presents the percentages of germination secured from tubers dug at regular intervals and stored for various intervals. The observations of the percentage of germination were made on March 24, at least three months after the last planting of tubers. Many of the tubers which had not germinated by that time had decayed.

Tubers dug on Sept. 27 when immature were viable if stored at a cool temperature for sufficient length of time. Tubers stored at least 35 days after digging germinated 100 per cent. Tubers dug at that time but stored a shorter time before planting failed to germinate. Tubers dug on Oct. 3 germinated 80 per cent with a week shorter storage period, that is, when stored only 28 days before planting. The later the harvest date, the shorter the storage period necessary before planting to secure 100 per cent germination. This does not take into account, however,

that germination was hastened where the storage period was longer at lower temperatures.

Tubers stored until Nov. 21 before planting germinated much more promptly than tubers stored for shorter periods, even though the tubers were of the same maturity when dug. Since the length of storage and the temperature of the storage influenced the speed of germination, an experiment was planned to determine the effect of storage temperature on the rest period and rate of germination.

TABLE 1. *Effect of maturity and length of storage period on the germination of artichoke tubers*

Date of harvest	Length of storage-days	Date planted	Percent-age germi-nation	Date of harvest	Length of storage-days	Date planted	Percent-age germi-nation
Sept. 27	7	Oct. 3	0	Oct. 17	7	Oct. 24	50
” ”	14	” 10	0	” ”	14	” 31	100
” ”	21	” 17	0	” ”	21	Nov. 7	80
” ”	28	” 24	0	” ”	35	” 21	100
” ”	35	” 31	100	” ”	65	Dec. 21	100
Oct. 3	7	” 10	0	Oct. 24	7	Oct. 31	100
” ”	14	” 17	0	” ”	14	Nov. 7	80
” ”	21	” 24	20	” ”	28	” 21	100
” ”	28	” 31	80	” ”	58	Dec. 21	100
” ”	35	Nov. 7	60				
Oct. 10	7	Oct. 17	0	Oct. 31	7	Nov. 7	0
” ”	14	” 24	80	” ”	14	” 14	50
” ”	21	” . 31	100	” ”	21	” 21	50
” ”	28	Nov. 7	100	” ”	51	Dec. 21	100
” ”	42	” 21	80				
” ”	72	Dec. 21	100				

Tubers were dug on Oct. 30, 1933, and placed in the following storage temperatures: —2 C., —0.5 C., 2 C., 4.5° C., 10° C. and 30° C. Since the periderm of the artichoke is very thin and there is a rapid loss of moisture at higher temperatures, it was necessary to store the tubers at the higher temperatures in dry sand to prevent excessive shriveling. All tubers at all storage temperatures were stored in dry sand. At semi-monthly intervals—the first and sixteenth of each month—tubers were removed from each storage, cut into one to one and one-half ounce pieces with at least two eyes and planted in four-inch pots. The pots were placed in a greenhouse at a temperature of about 15° C. The temperature fluctuated about three degrees in either direction. Tubers stored at —2° C. were held for 24 hours at 2° C. in order to thaw slowly. Records were kept on the date of the appearance of sprouts above ground. The results are presented in tables 2 to 8, inclusive.

In the tables, the number of days required before sprouts appeared above ground are expressed in columns headed 25, 50 and 75 per cent sprouting. The appearance of the first sprout is not a good index of the length of the rest period, because with certain treatments one tuber may

germinate but considerable time elapse before the germination of an-
other. Decay of tubers or the failure to secure 100 per cent sprouting
because of bud injury makes it undesirable to use complete germina-
tion as a comparative index.

TABLE 2.   *Tubers stored at seven different storage temperatures from Nov. 1 to Dec. 1*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece
	25 per cent	50 per cent	75 per cent	
—2.0	58 days	65 days	Failed to sprout	1.0
—0.5	68 ,,	74 ,,	,, ,, ,,	1.0
2.0	64 ,,	77 ,,	,, ,, ,,	1.0
4.5	74 ,,	79 ,,	,, ,, ,,	1.0
10.0	Failed to sprout			
24.0	,, ,, ,,			
30.0	,, ,, ,,			

TABLE 3.   *Tubers stored at six different storage temperatures from Nov. 1 to Dec. 15*

Storage temperatures °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Feb. 12
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	19 days	28 days	40 days	1.0	20.0
—0.5	30 ,,	34 ,,	35 ,,	1.0	16.5
2.0	31 ,,	38 ,,	43 ,,	1.0	11.9
4.5	35 ,,	39 ,,	45 ,,	1.0	10.2
10.0	Failed to sprout				
24.0	,, ,, ,,				

TABLE 4.   *Tubers stored at six different storage temperatures from Nov. 1 to Jan. 1*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Feb. 28
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	14 days	18 days	21 days	1.2	16.2
—0.5	20 ,,	21 ,,	24 ,,	1.0	17.2
2.0	20 ,,	26 ,,	32 ,,	1.0	13.2
4.5	27 ,,	31 ,,	34 ,,	1.0	13.9
10.0	35 ,,	41 ,,	47 ,,	1.0	6.7
24.0	Failed to sprout				

Tubers that were stored at a temperature of 10° C. or above from time of harvesting (Nov. 1) to Dec. 15 failed to germinate. Most of the tubers had rotted four months after planting. Although Loomis (12)

TABLE 5. *Tubers stored at five different storage temperatures from Nov. 1 to Jan. 15*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Mar. 15
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	13 days	15 days	16 days	1.6	21.5
—0.5	17 "	20 "	21 "	‵1.5	24.9
2.0	16 "	20 "	20 "	1.2	24.5
4.5	16 "	20 "	21 "	1.1	28.0
10.0	22 "	26 "	28 "	1.0	23.4

TABLE 6. *Tubers stored at five different storage temperatures from Nov. 1 to Feb. 1*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Mar. 24
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	12 days	12 days	13 days	1.6	22.8
—0.5	15 "	16 "	17 "	1.6	29.7
2.0	13 "	14 "	15 "	1.2	29.9
4.5	13 "	14 "	17 "	1.3	22.8
10.0	16 "	17 "	19 "	1.0	26.0

TABLE 7. *Tubers stored at five different storage temperatures from Nov. 1 to Feb. 15*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Apr. 1
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	11 days	13 days	14 days	2.0	19.9
—0.5	11 "	12 "	13 "	1.8	24.9
2.0	13 "	14 "	15 "	1.8	26.3
4.5	13 "	14 "	15 "	1.5	26.5
10.0	15 "	16 "	17 "	1.3	25.2

found that the rest period of potato tubers stored at 30° F. was shortened considerably, this temperature did not shorten the dormant period of the artichoke tubers. Tubers stored at this temperature failed to germinate when planted. It was impossible to store the tubers at this temperature for more than two weeks, even when the tubers were covered with sand, as excessive shriveling occurred. The lower the temperature at which the tubers were stored, the shorter the rest period until freezing temperatures were reached. Slight freezing hastened germination but heavy freezing was injurious to the tubers. Tubers

stored for only one month, at —2° C., required 58 days from planting for 25 per cent germination, but tubers stored for one and one-half months at the same temperature germinated 25 per cent in 19 days from planting, or just about one-third of the time. Tubers stored at 10° C. for one month and then planted, failed to germinate four months after planting, moreover, most of the tubers rotted; but when the tubers were stored for two months at the same temperature, they germinated 25 per cent in 35 days.

TABLE 8. *Tubers stored at five different storage temperatures from Nov. 1 to March 1*

Storage temperature °C.	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks Apr. 3
	25 per cent	50 per cent	75 per cent		
					inches
—2.0	12 days	14 days	15 days	2.0	25.2
—0.5	12 "	13 "	14 "	1.9	26.8
2.0	13 "	14 "	15 "	1.7	28.2
4.5	13 "	14 "	15 "	1.9	29.7
10.0	13 "	14 "	15 "	1.3	24.6

A record was kept of the average number of sprouts per seed piece which developed. Tubers stored at the lower temperatures (—2° C. and —0.5° C.) averaged a greater number of sprouts after two and one-half months in storage than tubers held at the higher temperatures. The tendency to develop a greater number of sprouts per seed piece increased at all storage temperatures as the storage period was prolonged and the number increased inversely with the temperature, that is, the lower the temperature and the longer the storage period, the greater the number of sprouts per seed piece.

Records were taken of the height of each individual plant about a month after germination. Tubers stored three and one-half months at —2° C. before planting, germinated on an average of four days before tubers stored at 10° C., but the plants grown from tubers stored at the higher temperature were taller one and one-half months after planting than those grown from tubers stored at the lower temperature.

In tables 9 to 13, inclusive, are presented the results of treating tubers with thiourea and ethylene chlorohydrin. When tubers were stored for one month at 4.5° C. and then immersed for one hour and two hours in various percentages of thiourea in water, germination was accelerated. Soaking the tubers in 4 and 6 per cent solutions for one hour cut the time required for germination in half. Soaking in 2 per cent solution for two hours was as effective as 4 and 6 per cent for one hour. Soaking tubers in a 10 per cent solution for either one or two hours caused a delay of about five days in germination. The above figures refer to germination of 25 per cent of the tubers.

Tubers stored at 2° C. for three and one-half months and then immersed in 2, 4 and 10 per cent solutions of thiourea for one and two hours did not germinate more quickly than check tubers not treated but stored at the same temperature. However, the tubers immersed in

4 and 10 per cent solutions produced more sprouts per eye than check tubers. Tubers immersed in 2 per cent solution did not produce any more sprouts than the check tubers. The average height of stalks one month after germination was higher with the check tubers than with the treated tubers, and tubers treated with the higher concentrations grew slower than tubers soaked in the solutions of lower concentration.

Table 9. *Tubers soaked in thiourea after one month in storage at 4.5° C. Planted Dec. 1*

Treatment	No. days from planting to sprouting			Ave. no. of sprouts per seed piece
	25 per cent	50 per cent	75 per cent	
2 per cent—1 hr.	33 days	36 days	41 days	1.0
4 " " —1 "	28 "	30 "	37 "	1.0
6 " " —1 "	28 "	30 "	36 "	1.0
10 " " —1 "	33 "	37 "	45 "	1.2
2 " " —2 hrs.	28 "	31 "	37 "	1.2
4 " " —2 "	28 "	28 "	34 "	1.0
6 " " —2 "	28 "	28 "	36 "	1.0
10 " " —2 "	33 "	37 "	45 "	1.2
Check	56 "	70 "	77 "	1.0

TABLE 10. *Tubers soaked in thiourea after two and one-half months in storage at 2°C. Planted Feb. 15*

Treatment	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks
	25 per cent	50 per cent	75 per cent		
2 per cent—1 hr.	12 days	12 days	14 days	1.9	21.8
4 " " —1 "	14 "	16 "	18 "	2.2	20.4
10 " " —1 "	17 "	19 "	21 "	4.0	16.4
2 " " —2 hrs.	13 "	14 "	15 "	1.8	24.2
4 " " —2 "	13 "	14 "	17 "	2.4	19.6
10 " " —2 "	15 "	16 "	18 "	5.0	11.6
Check	13 "	14 "	15 "	1.8	26.3

Tubers were also immersed in solutions of ethylene cholorhydrin. Concentrations of 2, 4, 6 and 10 cc. per liter were used and the tubers soaked for one and two hours after storage for one month at 4.5° C. The results were similar to those secured with thiourea, although thiourea caused sprouting on an average five days earlier than ethylene chlorohydrin. To secure 25 per cent germination the strength of the solution did not seem to affect the rate of germination, but to secure 75 per cent germination 10 cc. per liter was more effective than weaker concentrations of the solution.

Another series of treatments was used on tubers stored for one month at 4.5° C. The tubers were cut into pieces ready for planting. The cut tubers were dipped in concentrations of 10, 15, 30, 45 and 60 cc. per liter of ethylene cholorhydrin. The seed pieces were placed in glass quart jars. The solution was then poured in until the jar was full and

TABLE 11. *Tubers stored one month at 4.5° C. and then treated with ethylene chlorohydrin. Planted Dec. 1*

Treatment	No. days from planting to sprouting			Ave. no. of sprouts per seed piece
	25 per cent	50 per cent	75 per cent	
2 cc. per liter— 1 hr.	33 days	65 days	70 days	1.0
4 cc. ,, ,, — 1 ,,	33 ,,	43 ,,	70 ,,	1.0
6 cc. ,, ,, — 1 ,,	37 ,,	44 ,,	73 ,,	1.0
10 cc. ,, ,, — 1 ,,	30 ,,	35 ,,	37 ,,	1.0
2 cc. ,, ,, — 2 hrs.	36 ,,	54 ,,	73 ,,	1.0
4 cc. ,, ,, — 2 ,,	35 ,,	65 ,,	70 ,,	1.0
6 cc. ,, ,, — 2 ,,	30 ,,	35 ,,	46 ,,	1.0
10 cc. ,, ,, — 2 ,,	24 ,,	28 ,,	43 ,,	1.0
Check	56 ,,	70 ,,	77 ,,	1.0
10 cc. per liter—48 hrs.	32 ,,	36 ,,	38 ,,	1.0
15 cc. ,, ,, —48 ,,	23 ,,	30 ,,	32 ,,	1.0
30 cc. ,, ,, —48 ,,	18 ,,	23 ,,	28 ,,	1.0
45 cc. ,, ,, —48 ,,	19 ,,	25 ,,	33 ,,	1.0
60 cc. ,, ,, —48 ,,	23 ,,	25 ,,	34 ,,	1.0
5 cc. ,, ,, —72 ,,	41 ,,	70 ,,	75 ,,	1.0
10 cc. ,, ,, —72 ,,	27 ,,	34 ,,	50 ,,	1.0
15 cc. ,, ,, —72 ,,	21 ,,	29 ,,	38 ,,	1.0
20 cc. ,, ,, —72 ,,	20 ,,	28 ,,	41 ,,	1.0
30 cc. ,, ,, —72 ,,	19 ,,	34 ,,	dead	1.0

TABLE 12. *Tubers stored at 2° C. for two months, then treated with ethylene chlorohydrin, stored again at 2° C. and planted at weekly intervals*

Treatment	No. days from planting to sprouting			Av. ht. stalks at one month
	25 per cent	50 per cent	75 per cent	
Treated—stored 1 week	19 days	20 days	24 days	12
Check	16 ,,	16 ,,	19 ,,	10
Treated—stored 2 weeks	17 ,,	19 ,,	22 ,,	12
Check	16 ,,	17 ,,	19 ,,	14
Treated—stored 3 weeks	15 ,,	19 ,,	22 ,,	6
Check	14 ,,	15 ,,	22 ,,	13
Treated—stored 4 weeks	15 ,,	28 ,,	———	6
Check	14 ,,	16 ,,	17 ,,	14
Treated—stored 5 weeks	———	———	———	
Check	14 ,,	16 ,,	17 ,,	15

TABLE 13. *Tubers stored at 2° C. three months, then at 24° C.*

Treatment	No. days from planting to sprouting			Ave. no. of sprouts per seed piece	Ave. ht. stalks at one month
	25 per cent	50 per cent	75 per cent		
					inches
2 weeks at 24° C.	13 days	15 days	16 days	1.7	23.5
Check	13 ,,	14 ,,	15 ,,	1.8	25.3
4 weeks at 24° C.	14 ,,	15 ,,	16 ,,	1.5	24.6
Check	13 ,,	14 ,,	15 ,,	1.7	28.2

then poured off; the tubers were shaken to remove the excess liquid and the jar sealed for 48 hours and then the tubers were planted. A similar experiment was performed using 5, 10, 15, 20 and 30 cc. of ethylene chlorohydrin per liter and the tubers sealed in the jars for 72 hours before planting.

Tubers that had been soaked in a solution of 30 cc. per liter for 48 hours sprouted more promptly than tubers treated with other concentrations. Nearly 50 per cent of the tubers treated with 30 cc. per liter and sealed for 72 hours failed to germinate. None of the ethylene chlorohydrin treatments caused multiple sprouting.

Tubers stored at 2° C. for two months were treated with ethylene chlorohydrin, dipping them in a 30 cc. per liter solution and sealing for 48 hours as in the previous experiment. The tubers were washed with tap water after removal from the jars and stored again at 2° C. At weekly intervals 20 pieces were planted and 20 pieces stored at 2° C., but untreated. The treated tubers germinated more slowly than the check tubers and after five weeks of storage the treated tubers failed to germinate and rotted in the soil in less than a month after planting. The stalks of treated tubers grew at the same rate after two weeks' storage, but tubers stored three and four weeks produced stalks that grew at about one-half the rate of growth of the check tubers. Tubers stored at 24° C. immediately after harvesting, failed to germinate, so an experiment was run to determine the effect of this temperature on germination where the rest period was about over. Tubers which had been stored at 2° C. for three months were placed at 24° C. for two and four weeks and then planted. This had no effect, apparently, on the rate of germination, growth rate of stalks, nor on the average number of sprouts per seed piece.

Sodium nitrate had no effect on shortening the rest period. Tubers were soaked for one and two hours in 2, 4, 6 and 10 per cent solutions. Rosa (16) found sodium nitrate somewhat effective on potatoes.

Dormant tubers were exposed to temperatures of —15° C. and —20° C. for two weeks, but the tubers failed to grow when planted, although the tubers were thawed slowly at 2° C. before planting.

Tubers stored for one month at 10° C. which had failed to grow when planted for two months were placed at a temperature of —2° C. These tubers were planted in four-inch pots. The potted tubers were exposed to —2° C. for one week. The tubers sprouted in two weeks after returning to the greenhouse.

DISCUSSION

Tubers harvested on Sept. 27 germinated as promptly as tubers harvested Nov. 1 if stored for the same length of time at the same temperature. The tubers dug at the earlier date were smaller than those dug later and no doubt less mature, but maturity apparently does not affect germination. Tubers dug on Nov. 1 were from plants that were quite green until the foliage was killed by heavy freezing two days earlier. In this latitude the tops of the plants do not die until killed by frost, so that all tubers are dug from immature plants. The plants do not bloom until late September or October, so that all tubers might be classified as immature.

. Storage of the artichoke tubers slightly below the freezing point shortens the dormant period of the tubers. The factors which cause this are for the most part unknown. Periods of exposure just below the point of injury may cause stimulation of the tissue into renewed activity. Coville (3) suggests, in the case of plants subjected to low temperature, weakening of the plant membranes and allowing greater rates of diffusion of enzymes or food material. Appleman (1) found that oxygen supply to the tissue was a critical factor in experiments with the potato. He was unable to correlate the rest period with enzyme changes.

According to Loomis (13), various resting plant organs have been found to pass through their normal rest period in minimum time at temperatures either 15° C. above or 15° C. below normal (20° C.). Successful treatments have been accompanied by similar chemical responses in the storage tissues, whether due to high or low temperatures or chemical treatments, and have been characterized by accumulations of available carbohydrates, particularly sucrose, in the treated tissues. However, Denny (8) points out that the increase in sugar and decrease in starch may be a result of breaking the dormancy and not the cause of it.

Denny, Miller and Guthrie (9) studied catalase, peroxidase and oxidoreductase activity in chemically treated potato tubers but make no claim that the changes in enzyme activity are to be looked upon as the cause of the growth of buds or as furnishing proof of the causes of the previous state of dormancy.

Thiourea solutions caused the growth of two or more buds from a single eye of the tubers. At each eye or bud of the tubers there are the rudiments of several buds. Usually only one bud will grow, the growth of the rest of the accessory buds being inhibited. If the sprout which starts growth first is removed, other buds may become active. Treating the tubers with thiourea solutions when the rest period is over, or nearly so, has more effect on multiple sprouting than treatment when the tubers are quite dormant. More sprouts per seed piece were produced on tubers stored at lower temperatures than at the higher temperatures studied. However, usually more than one produced a sprout, while thiourea caused multiple sprouting of a single eye. The growth of more than one sprout from a single eye results in spindly sprouts. Since thiourea had more effect on multiple sprouting when the rest period was nearly over than when the tubers were quite dormant, and since more sprouts per seed piece were produced on tubers stored at lower temperatures than at the higher temperatures, injury to the primary bud in the eye may be the cause of multiple sprouting. Ethylene chlorohydrin and thioureau treatments delayed germination when applied to tubers no longer dormant; this result also suggests injury to the eye or bud. Loomis and Evans (14) found that onions could be forced in the early part of the rest period by splitting combined with forced injections of tap water. Water soak treatments were not used in these experiments, but since the sodium nitrate soak treatments were not effective in shortening the rest period, it is probable that tap water would not be effective.

SUMMARY

Experiments are reported in which the rest period of dormant tubers of the Jerusalem artichoke was shortened by storage at temperatures near or slightly below freezing. The length of the rest period or dormant

stage was directly dependent on storage temperature; the lower the temperature down to slightly below freezing, the shorter the rest period.

Ethylene chlorohydrin and thiourea were somewhat effective in shortening the rest period. Sodium thiocyanate was not so effective as ethylene chlorohydrin and thiourea while sodium nitrate was not effective at all.

Tubers harvested on Sept. 27 germinated as promptly, although considerably less mature than tubers harvested on Nov. 1.

Thiourea caused multiple sprouting, which was more pronounced when tubers were treated after dormancy had been broken.

Tubers held for three or more months at temperatures near the freezing point exhibited a tendency to produce more than one sprout per seed piece.

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NOTES ON THE BIOLOGY OF ONCOPELTUS FASCIATUS (DALLAS)

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Several years ago the writer became interested in a large black and red lygaeid *Oncopeltus fasciatus* (Dallas) (Pl. I, A). which occurs quite frequently on milkweed in Iowa. Upon investigating the literature, it was found that very little is recorded concerning the life and habits of this insect under field, greenhouse or controlled conditions of temperature and relative humidity. The ability of this insect to live and reproduce normally when feeding on dried milkweed seeds and supplied with water, makes it a very desirable form to study throughout the year.

CLASSIFICATION, HISTORY AND DISTRIBUTION

Oncopeltus fasciatus (Dallas) belongs to the group of insects commonly called "true-bugs," of the order Hemiptera. It is placed in the family Lygaeidae, which is widely known as the "chinch-bug" family. Although many publications mention this species, little information is given in the literature concerning its life and habits. Collecting records and field observations constitute the bulk of published material.

Strangely enough, the first description of *Oncopeltus fasciatus* to be recorded in literature was, through a mistake in identification by Herrich-Schaeffer (1842), supposedly a description of Fabricius's *Lygaeus aulicus*. Dallas, however, in 1852 recognized from Herrich-Schaeffer's description and figure a new form which he named *Lygaeus fasciatus*. In this same notation, Dallas states that the species was represented in the British Museum by specimens from the United States, Brazil, Colombia and British Guiana. Numerous records, which have greatly widened its known distribution, have since been recorded in the literature; and it may now be considered one of the most widely distributed species of Hemiptera in the Western Hemisphere.

FOOD

As the vernacular name "milkweed bug" implies, *Oncopeltus fasciatus* (Dallas) feeds on numerous species of milkweed plants. Eggs, nymphs and adults were collected from the flowers and pods of common milkweed, *Asclepias syriaca* L., nymphs in the third and fourth instars from pods of the whorled milkweed, *Asclepias verticillata* L., and several mating pairs from the showy milkweed, *Asclepias speciosa* Torr., during the summer of 1932.

Both nymphs and adults will suck the juices from grasses in an effort to secure moisture. When wheat or bluegrass was grown in the rearing

cages, the bugs grew to maturity on dried milkweed pods and seeds without taking any water other than that obtained from the plants. Neither the wheat nor the bluegrass in itself was a sufficient diet, because all efforts to rear the insects on grass alone failed.

METHODS

The experiments presented in this paper have been conducted under (1) constant conditions of temperature and relative humidity, (2) in an out-of-doors screened laboratory, and (3) under greenhouse conditions. Constant temperature machines (Brindley and Richardson, 1931) were used to obtain the desired temperatures, and the relative humidities were kept constant by using saturated solutions of certain inorganic salts. The humidity was maintained at approximately 70 per cent by keeping pans full of a saturated solution of sodium chloride placed directly in the air currents from the fan. This relative humidity was used in all the studies made except in determining the incubation period of eggs.

The eggs were obtained from adults confined in breeding cages, which were merely medium-sized lamp chimneys with a piece of cheese cloth fastened over each end by means of a rubber band. These bugs were fed on dried milkweed pods which had been collected in the fall before the seeds had escaped. The females laid their eggs in pieces of cotton placed in the cages for that purpose. At least once a day, or oftener when an experiment required an accurate record of the time the eggs were laid, the cotton was taken out and the eggs removed by pulling it apart, as the female generally inserted her eggs some distance into the cotton. The eggs were then put into other cages or stender dishes and these were placed in the desired conditions. Nymphs were reared in these cages or in stender dishes with ground glass tops. Circular pieces of paper toweling were placed in the bottom of the stender dishes and moisture was supplied daily.

In order to determine the length of time required for incubation, the eggs were placed in 10 mm. by 50 mm. shell vials. These were placed in 20 mm. by 80 mm. vials containing a saturated solution of the salt, giving the desired relative humidity and tightly corked. The vials were aerated once a day.

DESCRIPTIONS

EGG

Egg elongate-oval, more broadly rounded at cephalic end, nearly twice as long as broad, circular in cross-section, without distinct sculpturing or color markings on chorion; cephalic end with indication of circular lid or cap, the latter surrounded by 11 to 13 fairly prominent chorial processes, which are short, slightly bent inward, strongly constricted at their bases, and enlarged and somewhat rounded at their apices. Freshly deposited eggs chrome lemon-yellow, gradually changing to reddish, the red eyes and general form of the embryonic insect becoming quite distinctly visible beneath the chorion as incubation progresses. Size (average of 150 eggs): Length, 1.41 mm.; width, 0.63 mm.

NYMPHAL INSTARS

The spotted milkweed bug passes through five distinct nymphal instars in its growth to the adult. Each of these instars is quite individual

and displays characters that make it easily differentiated. A short description of each instar follows.

First Instar. Elongate oval, slightly larger than egg. Widest across middle portion of abdomen. Tapers gradually toward head. Posterior portion of abdomen slightly rounded. Head triangular, obtusely rounded in front. Antennae linear, finely pubescent, about three-fourths as long as body, inserted laterally, four segmented, the proportional length of the segments—I: II: III: IV: : 3: 7: 7.1: 12. Eyes reddish, lateral, prominent. Prothorax one and one-half times as long as wide, anterior margin concave, sides widening to posterior. Abdomen widest at segment IX, sides slightly rounded. Rostrum four-segmented, reaching beyond posterior end of abdomen. Legs finely pubescent, tarsi two-segmented. Legs, antennae and rostrum pale smoky brown. Joints of antennae between segments and last half of distal segment lighter. Head and thorax much darker, the latter with a rather broad median reddish-orange stripe. Abdomen reddish-orange, more yellowish at base. Width across widest portion of abdomen, 1.1 mm. Length, 3.1 mm.

Second Instar. Slightly darker than first, joints between antennal segments and legs darker. Antennal segments in the proportion 5: 11: 10: 17. Width across widest portion of abdomen, 1.210 mm. Length, 3.502 mm.

Third Instar. In this instar the nymph begins to show wing-pads which appear as swellings on the posterior margin on both sides of the metathorax. A darker color is prevalent in this stage than is found in either of the previous. Antennal segments in the proportion 6: 18: 16: 25. Width across widest portion of abdomen, 1.362 mm. Length, 4.166 mm.

Fourth Instar. Mesothoracic wings appear in this stage and nearly cover the metathoracic pair. Antennae, head, wings and legs are black. Body and margins of wing-pads covered with fine pubescence. Two spots or stink gland holes on the third and fourth abdominal segments are more prominent than in any previous stage. Antennal segments in the proportion 8: 26: 22: 34. Width across widest portion of abdomen, 2.017 mm. Length, 6.211 mm.

Fifth Instar. Elongate-oval, widest across middle of abdomen, tapering gradually toward head; posterior portion of abdomen broadly rounded. Head triangular, obtusely rounded in front, slightly rugose, with more or less pronounced dorso-median ridge broadly rounded, extending from apex to middle portion posterior to which is a slight curved transverse depression. Antennae linear, finely pubescent, about half as long as body, inserted laterally, four-segmented, in the proportion 12: 38: 32: 46. Eyes lateral, prominent. Prothorax almost twice as wide as long, widened posteriorly, the anterior margin concave, the sides straight, gradually widening to posterior margin of third abdominal segment. Lateral margins of wing-pads slightly rounded. Sides of pronotum and abdominal segments slightly rounded. Rostrum four-segmented, reaching beyond posterior margin of thorax. Legs finely pubescent, comparatively long and slender. Antennae, legs, rostrum, upper portion of head, posterior margin of prothorax, lateral spots on all of the abdominal segments and median dorsal spot on the fifth, sixth, seventh, eighth and ninth abdominal segments, and apex of ventral abdominal surface black. Remainder of body reddish-orange. Length at the end of this instar varies from 9 to 11 mm. Width across widest portion of abdomen, 3.44 mm.

Adult. Elongate-oval. Color black and red; cheeks, side margins of

pronotum, basal and apical thirds of elytra usually reddish, sometimes fading to reddish orange. Apex of scutellum, coxae and abdomen for the most part, reddish yellow. Legs and antennae shining black. Genital plates, spots near middle of third and fourth ventrals and front angles of each ventral at sides black. Pronotum declivent in front, deeply impressed at each side. Antennal segments in the proportion 15:45:38:65. Width across widest portion, 4.8 to 6.0 mm. Length, 13 to 18 mm.

TABLE 1. *Table of measurements*

	Stage of development					
	1st	2nd	3rd	4th	5th	Adult
Width across eyes	.511	.688	.946	1.290	1.706	2.193
Antennal I	.129	.215	.258	.344	.516	.645
Antennal II	.308	.473	.795	1.118	1.634	1.935
Antennal III	.301	.430	.688	.946	1.376	1.634
Antennal IV	.516	.738	1.079	1.462	1.988	2.709
Total length of antenna..............	1.254	1.856	2.820	3.870	5.514	6.923
Total length of rostrum	1.548	2.494	3.407	4.558	6.020	7.310
Width greatest	1.102	1.210	1.362	2.017	3.441	4.8–6.3
Length	3.114ʰ	3.502	4.166	6.211	9–11	10–18

LIFE HISTORY AND HABITS

INCUBATION OF EGG

Certain definite macroscopic changes accompany the development of the egg. Within a comparatively short period of time, ranging from six to twelve hours—depending largely on the temperature—the incubating egg gradually darkens and turns reddish in color. At a constant temperature of 34.5° C. the egg changes to a reddish hue in 12 hours and the eye spots become visible in 24 hours; at 29.5° C. it turns reddish in 18 hours and eye spots show in 36; and at 24.5° C. reddening is noticeable in 36 hours and eye spots become evident in about 60 hours. Hatching takes place at the end of 72 hours at 34.5° C., in 96 hours at 29.5° C., and in 144 hours at 24.5° C.

A short time before hatching, the form of the embryonic insect can be seen through the translucent shell-membrane with the aid of a good lens. Faint striations mark the sutures separating the abdominal segments and the head and thorax of the embryo. Two deep red pigmented spots near one pole mark the location of the eyes. The chorion, or shell, is thin and easily broken.

The egg is very susceptible to the influence of external factors, especially temperature and moisture. High temperatures, within limits, accelerate development, whereas low temperatures retard incubation. (See table 2.) Eggs placed in dry sand immediately begin to desiccate and soon lose much of their moisture content, shrivel and fail to hatch. Conversely, an excess of water in the sand interferes with development and in numerous instances prevents hatching.

TABLE 2. *Incubation period of eggs*

Temperature	Relative humidity	No. of eggs	Hatched		Incubation period. Days
			Number	Percentage	
24.5° C.	75%	100	100	100	6
24.5° C.	33%	100	100	100	·6
29.5° C.	75%	100	100	100	4
29.5° C.	33%	100	100	100	4
34.5° C.	75%	100	100	100	3
34° C.	33%	30	20	66.6	3
38.0° C.	75%	25	0	0	—
Room	75%	30	30	100	6
Room	33%	30	30	100	6
Outdoors	75%	30	30	100	6
Outdoors	33%	30	30	100	6

HATCHING

At the moment of hatching, the cephalic end of the eggshell is irregularly longitudinally ruptured by pressure on the shell membrane by the nymph. An examination of several hundred egg shells after incubation failed to show in a single case where the rupture had taken place in the form of the circular lid or cap as indistinctly outlined on the external surface of the chorion. From 10 to 25 minutes are required for the insect to escape from the shell. It is quite interesting to observe the hatching nymph slowly unfold its appendages, the order usually being antennae, first pair of legs, second pair of legs and third pair of legs. After a period of from 10 to 20 minutes, the newly hatched nymph begins to move slowly about and soon starts feeding.

FEEDING HABITS OF THE NYMPHS UNDER CONTROLLED CONDITIONS

Soon after hatching, the nymphs begin moving in search of food. Upon finding a milkweed pod, one would stop, raise the anterior part of its body, and then explore the surface with its beak. As soon as a favorable spot was found, the beak would be inserted. Then the nymph, after feeding for several minutes, would remove its beak, crawl off the pod and seek water.

For food, dried milkweed pods and their seed furnished the sole diet, and a vial of water plugged with a wad of cotton furnished the water. It was necessary to provide water to rear or keep the milkweed lygaeid alive on seeds or pods of the milkweed plant.

HABITS OF NYMPHS IN THE FIELD

During the summers of 1932 and 1933 the writer made frequent trips to various patches of milkweed in the vicinity of Ames, Ottumwa, New Sharon, Iowa City and a few other points to observe the habits of this

insect under natural conditions. Nymphs began to make their appearance in the field during the last week in July. They are somewhat gregarious in habit, and it was not uncommon to find large numbers feeding on a single milkweed pod. Unless approached with caution, the whole group would fall to the ground and not infrequently "play possum" for several minutes.

ECDYSIS

A few hours before molting, usually within two or three, the nymph stops feeding and moving about and becomes very quiet. Gradually the body assumes a slightly swollen appearance, and a light colored longitudinal line appears along the median dorsal parts of the head and thorax. In a few minutes the body begins to contract spasmodically until the cuticula ruptures along this light line and the insect within may slowly work itself out of its old "skin." During the molting period the nymph is in a very helpless condition and at the mercy of other members of the same species or other insects with cannibalistic propensities. Molting takes place on the under side of milkweed leaves in the field, thus avoiding the direct rays of the sun.

LENGTH OF INSTARS

The length of a stadium depends primarily upon temperature, humidity and food conditions. Nymphs are active, and growth is greatly accelerated by high temperatures. In the accompanying tables 3 to 6 and figures 1 to 5, the effect of temperature upon the rate of development from the time the eggs are laid until the adult stage is reached is illustrated.

TABLE 3. *Summary of life-history at constant temperature of 34.5° C. and relative humidity of 70 per cent*

Females (12 individuals)

	Stage						Total days		
	Egg	1st	2nd	3rd	4th	5th	Nymphal Life	Adult Life	Entire Life
Average days....	3.00	5.00	5.00	4.86	5.71	7.28	30.85	27.93	58.78
Range in days	—	—	—	4–5	5–7	6–9	30–32	24–32	56–63

Males (16 individuals

Average days....	3.00	5.00	5.00	5.06	4.87	7.06	29.99	29.44	59.43
Range in days	—	—	—	4–6	5–6	5–8	29–32	28–34	57–63

Males and Females (30 individuals)

Average days......	3.00	5.00	5.00	4.90	5.76	7.10	30.76	28.37	59.13
Range in days	—	—	—	4–6	5–7	6–9	29–32	24–34	56–63

TABLE 4. *Summary of life-history at constant temperature of 29.5° C. and relative humidity of 70 per cent*

Females (30 individuals)

	Stage						Total days		
	Egg	1st	2nd	3rd	4th	5th	Nymphal Life	Adult Life	Entire Life
Average days......	4.00	5.83	5.90	6.06	4.46	6.83	33.07	32.72	65.80
Range in days	——	5–7	5–7	5–8	4–8	5–8	33–37	22–35	56–71

Males (40 individuals)

Average days......	4.00	5.90	6.07	6.02	6.00	8.35	36.34	34.53	70.87
Range in days	——	5–7	5–8	6–7	——	7–10	34–38	29–39	65–76

Males and Females (70 individuals)

Average days	4.00	5.87	6.57	6.04	6.11	7.55	36.14	32.56	68.70
Range in days	——	5–7	5–8	5–8	4–8	5–10	33–38	22–39	56–76

TABLE 5. *Summary of life-history at constant temperature of 24.5° C. and relative humidity of 70 per cent*

Females (17 individuals)

	Stage						Total days		
	Egg	1st	2nd	3rd	4th	5th	Nymphal Life	Adult Life	Entire Life
Average days	6.00	7.00	7.05	6.41	6.35	11.00	43.81	30.72	74.53
Range in days	——	6–8	7–8	6–7	6–7	9–14	42–46	28–35	71–78

Males (13 individuals)

Average days	6.00	7.07	7.00	6.39	5.92	10.46	42.84	34.36	77.30
Range in days	——	6–8	——	6–7	5–7	9–13	41–45	26–39	70–80

Males and Females (30 individuals)

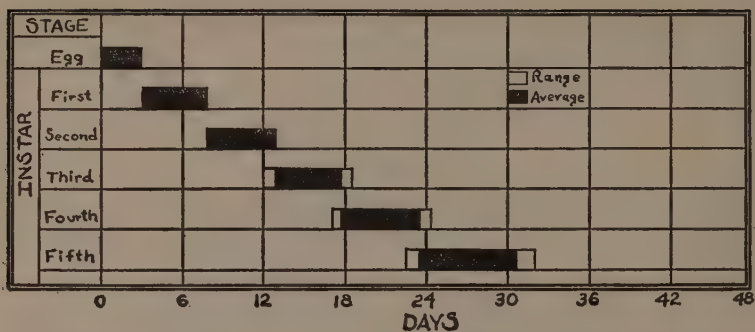| Average days	6.00	7.00	7.03	6.40	6.23	10.73	43.39	32.34	75.73
Range in days	——	6–8	7–8	5–7	5–7	9–14	41–46	26–39	70–80

Fig. 1. Summary of life-history at 34.5° C. and 70 per cent relative humidity.
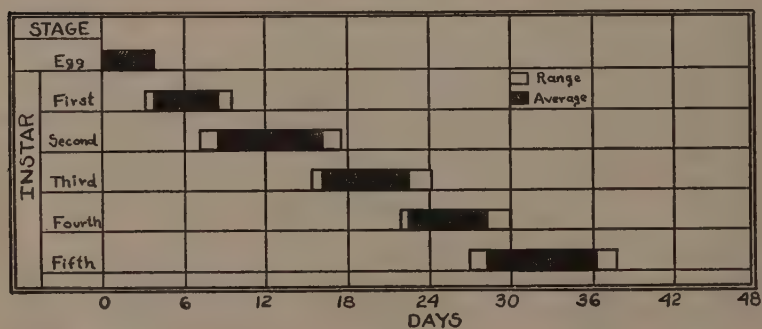

Fig. 2. Summary of life-history at 29.5° C. and 70 per cent relative humidity.
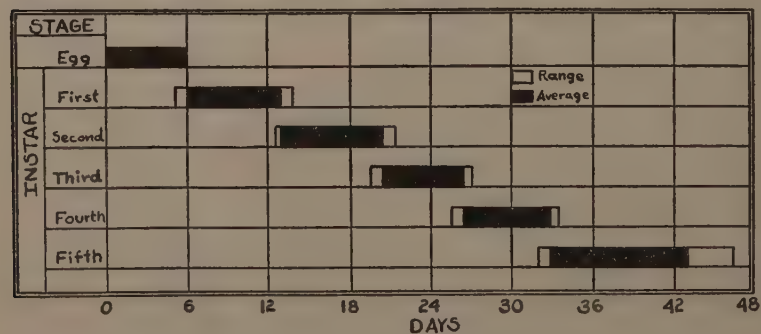
Fig. 3. Summary of life-history at 24.5° C. and 70 per cent relative humidity.

TABLE 6. *Summary of life-history in out-of-doors, screened laboratory* Females (13 individuals)

	Egg	1st	2nd	Stage 3rd	4th	5th	Total days
Average days	6.00	8.07	6.61	6.00	7.07	12.92	46.67
Range in days	—	7–9	5–7	—	6–10	10–15	39–48

Males (17 individuals)

	Egg	1st	2nd	Stage 3rd	4th	5th	Total days
Average days	6.00	8.11	6.70	6.00	6.17	12.00	44.98
Range in days	—	7–9	6–8	—	5–10	11–13	40–48

Males and Females (30 individuals)

	Egg	1st	2nd	Stage 3rd	4th	5th	Total days
Average days	6.00	8.10	6.67	6.00	6.90	12.40	46.07
Range in days............	—	7–9	5–8	—	5–10	10–15	39–48
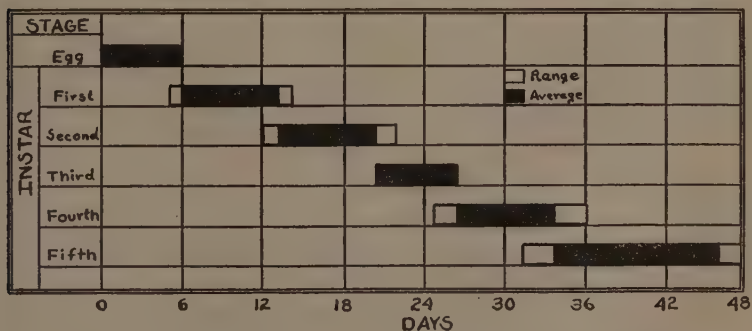
Fig. 4. Summary of life-history in out-of-door laboratory.

THE EFFECT OF FOOD UPON RATE OF GROWTH

Two types of food, namely, (1) growing milkweed plants and (2) dried seeds of milkweeds, were used in rearing cages placed side by side in the greenhouse.

Nymphs in the first instar, immediately after hatching, were placed in each cage. Those in the cage supplied with growing milkweed for food were not supplied with water. In the other cages, the diet of the nymphs consisted of dried milkweed pods and water. All other conditions except food were kept as nearly identical as possible. Table 7 shows a comparison of the rate of growth of the two groups.

TABLE 7. *Comparison of development when fed on growing milkweed and ripe dried seeds of milkweed (water must be supplied with dried seeds)*

Food		Stage					Total days
		1st	2nd	3rd	4th	5th	
Green plants	Average days....	5.71	6.00	6.35	6.43	9.71	34.20
	Range in days....	5–6	5–7	5–7	5–7	8–11	
Dried Pods	Average days	5.26	6.00	5.86	5.86	8.73	31.71
	Range in days....	5–6	——	5–6	5–7	8–10	

Fig. 5. Comparison of life–history at 24.5° C., 29.5° C. and 34.5° 'C. and 70 per cent relative humidity.

ADULT

COPULATION

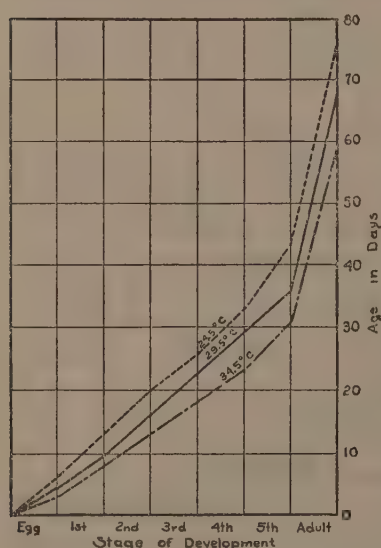After the last molt the adults feed for several days before reaching sexual maturity, the length of the pre-mating period greatly depending upon the temperature and food conditions. When reared at a constant temperature of 34.5° C., the pre-copulation period varied from 6 to 8 days, at 29.5″ C., from 7 to 13 days; and at 24.5° C., from 8 to 15 days. Dried milkweed pods were used as the sole source of food, and the relative humidity was kept at 70 per cent in each of the above cases.

The method of copulation is very similar to that of many other Hemiptera. The male usually approaches the female from one side, grasps her with his legs, and then attaches his genital organs. After the genital organs are firmly connected, the male loosens his hold with his feet and the bugs come to face in opposite directions. While *in coitu* the pair feed and move about on the plant, usually the female dragging the male. Normally copulation is repeated a number of times before egg-laying begins and may be repeated at numerous intervals during the entire period of egg deposition. The males are polygamic and the females polyandric. In the constant temperature cabinets the period of copulation lasted approximately 30 minutes at 38° C., 4 to 5 hours at 34.5° C., 12 to 30 hours at 30°., and for as long at 2 days at 24.5° C.

OVIPOSITION

Egg-laying begins from four to eight days after mating and may take place at any hour of the day or night. Under caged conditions the first

evidence of oviposition is noted by the female seeking a suitable place to deposit eggs. After finding a wad of cotton and exploring it with the rostrum and ovipostor, the female comes to rest and lays from 2 to 38 eggs, placing them well down in the cotton. Frequently the bug moves about considerably while in the act of laying, thus tending to place eggs here and there in the cotton.

When gravid females were confined in empty lamp chimneys, they failed to oviposit until after a suitable medium such as a piece of cotton or a fuzzy milkweed pod was added. In the field eggs may be deposited on the underside of the leaves of milkweed plants in irregular masses. As many as 30 eggs have been found on a single leaf.

Fig. 6. Mating, egg record, first oviposition and death of 30 females after reaching adult stage at 29.5° C. and 70 per cent relative humidity.

The largest number of eggs are laid during the early part of the adult stage (Fig. 6). In the case of 50 females which were reared at a constant temperature of 29.5° C. and a relative humidity of 70 per cent, 25 females laid the greatest number of eggs in the first batch. Of these 25 females only 2 laid eggs on the day following, whereas in all but one case they resumed laying on the second day following the first oviposition. Considerable variation is shown by individual females in the total number of eggs laid, the range being from as few as 5 to as many as 1238.

LONGEVITY OF ADULT

Very little is known regarding the duration of life, or "expectancy of life," of the members of the family Lygaeidae. Longevity in *Oncopeltus fasciatus* is somewhat variable, the males usually living slightly longer than the females. The total and mean length of adult life from the last nymphal molt to death at a constant temperature of 34.5° C. ranged from

24 to 34 days for females and from 28 to 34 days for males, the mean being 27.93 days and 29.44 days, respectively; at 29.5° C., 22 to 35 days for females and 29 to 39 days for males, the mean being 32.75 days and 34.46 days, respectively; and at 24.5° C. the range in the case of females was from 28 to 35 days and 26 to 39 days in males, the mean being 30.72 days and 34.46 days, respectively.

<div align="center">NATURAL ENEMIES</div>

Predators. Several species of predatory insects and a number of spiders frequently visit the milkweed plant in their quest for food. During the fruiting season the flowers and pods of milkweeds serve as suitable haunts in which predacious forms often secrete themselves. The ambush bug, *Phymata fasciata* (Gray), was encountered more frequently than any other insect preying on the larger milkweed lygaeid. On one occasion a female of a mating pair of phymatids was observed with its beak impaled in an adult. Two species of nabids, *Nabis ferus* (L.) and *Nabis rosepennis* Reuter, were taken a number of times while feeding on nymphs. A reduviid, *Sinea diadema* (Fab.), too, has not infrequently been found preying on both young and adults.

An orb-weaving spider very frequently spins its silken web on the milkweed plant, thus setting a treacherous trap for the larger milkweed lygaeid. Completely entangling the helpless bugs in the silken threads, the spider assures itself of a good supply of food. Nymphs as well as adults have been found on numerous occasions in the meshes of spiders' webs.

Cannibalistic Habits. Although *Oncopeltus fasciatus*—both as nymphs and adults—is primarily a plant-feeding form, it sometimes betrays certain cannibalistic tendencies. Weakened or injured individuals, and especially nymphs during the process of molting, often fall victims to their own relatives. As many as seven nymphs in the same rearing cage have been observed feeding on one of their members during ecdysis.

Parasites. *Oncopeltus fasciatus* is recorded as the host of both an insect and a protozoan parasite. It is the insect host of *Herpetomonas elmassiani* (Migone), a protozoon which is found also in milkweed. This particular organism has been recorded by Holmes (1925 c) as inhabiting the three-lobed thoracic salivary gland. He observed that they were definitely localized in this gland, colonizing only the dorsal and anterior lobes.

Morrill (1910) has reported a Tachina parasite of adults. This fly normally lays its eggs about the head and thorax.

<div align="center">HIBERNATION</div>

Although *Oncopeltus fasciatus* passes the winter in the adult stage in warm localities, such as California, the writer has been unable to find hibernating individuals in any stage in Iowa. Frequently adults of the genus Lygaeus and other related forms have been encountered, but the earliest record for Oncopeltus at Ames is July 7. Two cages were set up in a field over milkweed plants in the fall of 1932, and 50 adults and numerous nymphs were placed in each. The following spring all were dead. A rather interesting fact which developed from the examination of numerous specimens collected in various localities in Iowa over a period of years is that those specimens collected south of Ames along the Missouri border were often recorded as much as two weeks earlier during

the summer, while those collected north always appeared later than at Ames.

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PLATE I

Fig. A. Adult female of *Oncopeltus fasciatus* (Dallas).

Fig. B. Eggs on milkweed leaf shortly after deposition.

PLATE I

A

B
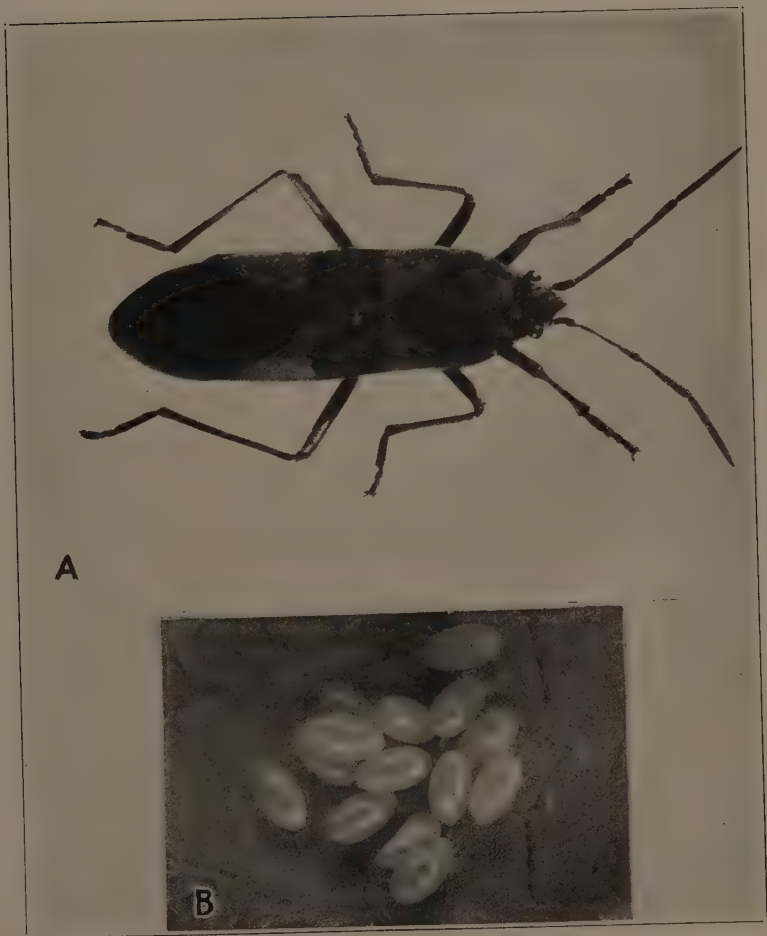
VANADIUM OXYTRICHLORIDE AS A SOLVENT II

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From the Department of Chemistry, Iowa State College

A previous paper[1] reports data on the solubilities of about 125 substances in vanadium oxytrichloride. Recently Mellor[2] has prepared a comprehensive review of the data and literature on vanadium oxytrichloride. The freezing point is given as "below —15°." No vapor pressures are given except at the boiling point. The data on the interrelations of the metals and vanadium oxytrichloride is meager and some of the statements are contradictory or incomplete. The reactions with the alkali metals and white phosphorus are instances.

This paper deals especially with the relationships between the elements and vanadium oxytrichloride, but includes some other data.

PREPARATION OF VANADIUM OXYTRICHLORIDE

The vanadium oxytrichloride was prepared by the method used by Briscoe and Little[3], when they were determining the atomic weight of vanadium, with one change in procedure. Much trouble was experienced in chlorinating the lower oxide obtained by passing hydrogen over hot vanadium pentoxide because of the caking of the oxide. The powdery reduced material became glassy and adhered to the walls of the glass tubes at temperatures below that required for chlorination. When the method of Briscoe and Little was modified by mixing the lower oxide with powdered charcoal, before its introduction into the tube for chlorination, no difficulty was experienced. It was then possible to use the high temperatures produced by Meeker burners and increase the rate of formation of the compound. The charcoal was never entirely consumed and may have been useful only in maintaining porosity. About 1,450 cc. of the crude product was prepared. After refluxing it over sodium and fractionally distilling it, about 900 cc. of a light yellow liquid whose analysis showed it to be 99.72 per cent $VOCl_3$ was secured. This was used in the experimental work reported in this paper.

EXPERIMENTAL

PHYSICAL PROPERTIES

The boiling point was 124.5° at 736 mm. pressure. The density was 1.854 at 0° and 1.811 at 32°. All attempts to freeze it failed. The lowest temperature tried was —77°. At low temperatures two phases appeared. One, the smaller in amount, was reddish in color and appeared to be a stringy clot of solid or extremely viscous substance.

[1]Brown and Snyder, J. Am. Chem. Soc., 47:2671 (1925).

[2]Mellor, J. N., A Comprehensive Treatise on Inorganic and Theoretical Chemistry. Longmans Green and Co., New York, 9:806 (1929).
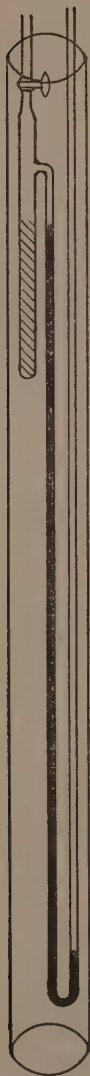
[3]Briscoe and Little, J. Chem. Soc., 105:1310 (1914).

The vapor pressure was determined over the range — 77° to + 80° and the boiling points at 736 mm. and at 760 mm. are known. Figure 1 is a drawing of the apparatus used. The open manometer, about 120 mm. in length, was filled to about one-half of its length with mercury. The bulb B was evacuated and permitted to refill with air slowly through a drying train five times. Approximately 25 cc. of freshly redistilled vanadium oxytrichloride was introduced into B through a tube which was drawn fine enough to pass through the stop-cock and reach to the bottom of the bulb. The vacuum pump was again attached to the tube above the bulb and evacuation continued until about one-half of the liquid had been evaporated at room temperature. During the evacuation the upper end of the manometer was cooled until liquid vanadium oxytrichloride formed above the mercury. Subsequent warming permitted the liquid to evaporate and its vapor to sweep the air from the manometer. After the air had been displaced in this manner, the tube was sealed off below the stop-cock. Readings of mercury level in the manometer were made with a cathetometer. The greatest error in determining the vapor pressure was due to the fact that vanadium oxytrichloride causes mercury to wet glass. It was found impossible to make satisfactory readings with decreasing pressure. Table 1 is a record of the data taken on vapor pressures.

TABLE 1. *Data taken on vapor pressures*

Temperature	Vapor pressure (mm.)	Temperature	Vapor pressure (mm.)
—77	4.1	40	60.9
—40	12.5	45	73.3
—15	17.5	50	89.5
— 5	20.6	55	98.3
0	21.0	60	125.4
7	23.9	65	139.2
10	32.8	70	149.3
15	34.2	75	176.2
20	37.2	80	222.3
25	41.9	85	270.0
30	47.8	124.5	736.0
5	56.5	127.16	760

Fig. 1. Apparatus for determination of the vapor pressure of vanadium oxytrichloride.

Figure 2 is a graph formed by plotting vapor pressure of vanadium oxytrichloride against temperature.

SOLUBILITIES OF ELEMENTS

The solubilities in and reactions of elements with vanadium oxytrichloride were determined by putting a small, carefully dried piece of each element which was available into about 5 cc. of the liquid. Observations were made for 10 to 15 days. Then

the temperature was raised to 125°. The following elements did not dissolve noticeably and did not show any indication of reaction under these conditions:

Aluminum	Molybdenum
Barium	Nickel
Beryllium	Nitrogen
Bismuth	Osmium
Boron	Oxygen
Cadmium	Phosphorus (red)
Carbon	Platinum
Cerium	Rhodium
Chromium	Ruthenium
Cobalt	Silicon
Columbium	Silver
Copper	Tantalum
Gold	Tellurium
Hydrogen	Thallium
Iridium	Tin
Iron	Titanium
Lead	Tungsten
Magnesium	Uranium
Manganese	Zinc
Misch Metal	Zirconium
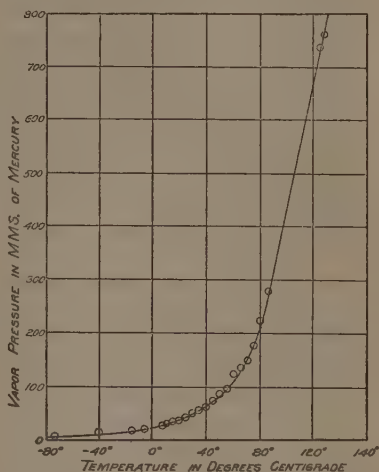
Fig. 2. Vapor pressure of vanadium.

The following elements dissolve with little or no reaction at room temperatures: Bromine, chlorine, iodine, mercury, selenium, sulfur and vanadium. The last four of this list may react slightly at room temperatures. Sulfur certainly does react at higher temperatures.

The quantitative solubility of iodine was determined at six points between 0° and 100°. A flask containing a supply of vanadium oxytrichloride was kept in contact with an excess of iodine for 12 to 15 hours at the chosen temperature. Then 5 cc. was pipetted into 250 cc. of water. After adding an excess of ferric alum and acidifying with sulfuric acid, the iodine was distilled into a solution of potassium iodide and titrated with thiosulfate. The solubility of iodine in vanadium oxytrichloride expressed as grams of iodine in 5 cc. of solution is reported in table 2.

TABLE 2. *Solubility of iodine in vanadium oxytrichloride*

Temperature degrees C.	Grams of iodine in 5 cc. solution		
	I	II	Average
0	0.0957	0.0820	.08885
25	0.4511	0.4237	.04374
47	1.2439	1.3123	1.27810
67	2,1872	2.2145	2.20085
92	3.1099	3.0416	3.07575
100	4.1935	4.2960	4.24475

Solubility of Arsenic

Arsenic reacts slightly with vanadium oxytrichloride. An excess of arsenic was added to a sample of vanadium oxytrichloride and let stand for some time, after which the arsenic in the supernatant liquid at 25° C. was determined.

0.6731 gms. contained 0.1485 gms. of arsenic

0.6569 gms. contained 0.1674 gms. of arsenic

These figures represent a solubility of 0.2206 gram and 0.2507 gram of arsenic per gram of vanadium oxytrichloride, respectively.

The following elements react with vanadium oxytrichloride at room temperature and in some cases with explosive violence: antimony, arsenic, caesium, calcium, gallium, indium, phosphorus (white), potassium, rubidium and sodium.

Nearly all investigators who have worked with this liquid have purified it by refluxing it over sodium. Brown and Snyder[4] reported that it may be refluxed over sodium for several days without suffering appreciable reduction or decomposition, that the metals potassium and sodium are unaffected at room temperature or at the boiling point of vanadium oxytrichloride, and that white phosphorus dissolves at room temperature without apparent reaction. In all three of these cases the report is misleading or entirely in error.

The statements regarding potassium and phosphorus were not accurately transcribed in condensing the original longer paper. Page 7 of Snyder's original thesis, on file in Iowa State College Library, contains the sentences: "Potassium reacts violently at about 90°." "White phosphorus dissolves without apparent reaction at room temperature but reacts vigorously at temperatures a little below the boiling point of water."

But these statements are not entirely correct. A bright piece of potassium darkens and collects a black deposit of what appears to be lower oxides of vanadium, from vanadium oxytrichloride, even at ordinary room temperature, and reacts explosively at about 100°.

Small pieces of white phosphorus do disappear in vanadium oxytrichloride without producing any residue or disturbance at room temperature. But large proportions of phosphorus produce rapid rises in temperature and violent and even explosive reactions, even when the contact between the two is effected at room temperature. Arsenic and antimony react with much less vigor than phosphorus, and bismuth does not react. The vigor of the reaction decreases with increasing atomic weight for these four elements.

An explosion occurred just before the distillation of a sample of vanadium oxytrichloride from sodium was completed. This led to closer observation of the interaction between this liquid and sodium. When the two were left in contact at room temperatures the bright metal became dull and later a noticeable black deposit appeared. Pieces of sodium and about 10 cc. of the liquid were sealed in a combustion tube and placed in a bomb furnace. The temperature was gradually raised. At 180°-185° the tube was shattered by an explosion. Lower oxides of vanadium and sodium chloride were recovered from the furnace. A second tube simi-

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[4]Brown and Snyder, J. Am. Chem. Soc., 47:2671 (1925).

larly filled was heated to 175° and cooled. Much of both sodium and vanadium oxytrichloride in it remained unchanged, but the sodium was covered with a layer of black substance.

The approximate temperatures of violent reaction between liquid vanadium oxytrichloride and the alkali metals are: caeseum 30°, rubidium 60°, potassium 100°, sodium 180°, lithium not determined. Calcium and indium react very slightly. Gallium reacts to form a blue precipitate.

REACTIONS WITH SULFUR DIOXIDE HYDROGEN SULFIDE AND GRIGNARD REAGENT

Liquid sulfur dioxide is immiscible and no reactions occur between these two substances in either the liquid or the gaseous state.

When liquid hydrogen sulfide and vanadium oxytrichloride are mixed a precipitate forms. One precipitate contained: vanadium 27.78 per cent, sulfur 20.14 per cent, and chlorine 33.08 per cent. The compositions of other precipitates varied, but were between that of the formulas for $VSCl_3$ and $VOCl . H_2S$, or $VOHSHCl$.[5]

When phenyl magnesium bromide and vanadium oxytrichloride were mixed no stable vanadium compound was formed. Vernon[6] reports the formation of a green organic compound by this method. The compound darkens at 85-100°, does not melt up to 220°, cannot be crystallized from any common organic solvent, burns in flashes of sooty yellow flame and leaves a residue containing vanadium.

SUMMARY

1. About 900 cc. of pure vanadium oxytrichloride were prepared by the action of chlorine on vanadium trioxide in the presence of finely divided charcoal. This method gave an excellent yield.

2. The freezing point was found to be at least 60° C. below that previously reported.

3. The vapor pressure from — 77° to + 80° was determined.

4. About 50 elements were found to be insoluble and non-reactive.

5. The quantitative solubilities of iodine and of arsenic were determined.

6. The reactions of sodium, potassium, rubidium, caesium, phosphorus, arsenic and antimony were studied.

7. The compound reacts with liquid hydrogen sulfide, but does not react with liquid sulfur dioxide.

8. It does not form stable vanadium organic compounds with the phenyl magnesium bromide.

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[5]Reactions of Inorganic Compounds with Hydrogen Sulfide. Harlan Preson Guest. Iowa State College J. Sci., 8, No. 1, 197-198 (1933).
[6]C. C. Vernon, J. Am. Chem. Soc. 53:3831 (1931).

THE STORAGE BEHAVIOR OF APPLES AS INFLUENCED BY NITROGEN FERTILIZATION AND STORAGE TEMPERATURE[1]

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The effect of the application of nitrogenous fertilizers to apple trees on the chemical make-up of the fruit and its metabolic activity, and the relationship of these to keeping quality in storage, is a subject of acute controversy in the fruit growing and storage industries. The use of nitrogen carrying fertilizers is frequently questioned when apples which have received such treatment break down prematurely, and it is assumed that the treatment predisposes the fruit to breakdown or other related diseases. Such an assumption has some justification on the basis that the results of certain research work have led to the conclusion that the nitrogen content of the apple may regulate metabolic activity in storage and that, therefore, the proportion of nitrogen to the storage reserves in the form of carbohydrates and organic acids determines the storage life of the fruit. Much of the earlier work on apple storage was done before the use of fertilizing materials was adopted as a standard practice, and, since more fruit is being produced under a system which includes the application of nitrogenous fertilizers, the question of their effect on stored fruit is becoming of increasing importance.

In the work herein reported the writer has undertaken to determine whether there is a casual relationship between the nitrogen content of the apple and susceptibility to soggy breakdown; whether the relationship between the sugar and nitrogen content of the fruit is affected by sodium nitrate applications to the trees; and whether this relationship manifests itself in the susceptibility to soggy breakdown.

Archbold (2) and Kidd and West (11) found a correlation to exist between nitrogen content and respiratory activity in apples, and storage tests showed low nitrogen fruit to keep longer than high nitrogen fruit. Gourley and Hopkins (6) and Aldrich (1) found that nitrogenous fertilizers increased the nitrogen content of the apple, but not its susceptibility to breakdown in storage. Degman (4), Weinberger (19) and Knowlton and Hoffman (12) found no correlation to exist between nitrogen fertilization and storage quality in apples. The results of Overley and Overholser (14), on the other hand, indicated that nitrogen fertilizers increased the susceptibility to Jonathan breakdown, particularly in seasons when the trouble was common. Harding (7) found a correlation between the nitrogen fertilization of Grimes apples and their respiratory activity.

SOURCE AND TREATMENT OF MATERIAL

The experimental fruit for the 1929-30 storage season, in the case of nitrated trees, was obtained from 25-year-old trees which had received

nitrate of soda applications in 1927, 1928 and 1929; in the case of the check trees, from trees of a nearby orchard which had never received fertilizer. For convenience, the orchard with the different nitrate treatments will be hereafter referrrd to as orchard A, and the one with only check treatments as orchard B. The fertilized orchard was located in a loess type of soil which is considered well adapted for fruit trees, while the check orchard lay in a gravelly type of soil of less uniform texture and was less retentive of moisture. Treatments in the fertilized orchard included trees which were not fertilized after 1928, so that in a sense it also contained check treatments. For the storage seasons of 1930-31 and 1931-32, the fruit was obtained from the same trees as before. These trees received the same treatment continually and comparable storage fruit was obtained from each treatment each year.

These treatments in the fertilized orchard varied in the nitrate applied each year. In treatment I, the four trees each received five pounds of nitrate in 1928; in treatment II, each received five pounds in 1927 and 1928; in treatment III, each received five pounds in 1927, 1928 and 1929; in treatment IV, each received five pounds in 1927 and 1928, and ten pounds in 1929. In 1930 and 1931, additional five and ten pound applications were given to treatments III and IV, respectively. Treatment I received no application in 1930 and 1931, while treatment II received a five pound application in 1931. The following tabulation shows the arrangement and status of each treatment for the three years of the experiment:

<center>Treatment status for different years</center>

Treatment	Orchard	1929	1930	1931
I	Fertilized	0–5–5	0–5–0–0	0–5–0–0–0
II	”	5–5–0	5–5–0–0	5–5–0–0–5
III	”	5–5–5	5–5–5–5	5–5–5–5–5
IV	”	5–5–10	5–5–10–10	5–5–10–10–10
V	Check	0–0–0	0–0–0–0	0–0–0–0–0

In the above table, the numerals 0, 5 and 10 indicate the nitrate fertilizer in pounds per tree applied in different years and do not refer to other mineral nutrients. The only fertilizer used in this investigation was nitrate of soda. For convenience, treatments I and II will be referred to as the minus nitrogen treatments; treatment III as the normal nitrogen treatment; treatment IV as the plus nitrogen treatment.

The storage treatment was comparable during each of the three seasons. Grimes and Jonathan apples were stored in standard boxes in duplicate lots at temperatures of 30-31° F., 35-36° F. and 48-50° F. Lots given immediate treatment were stored the day of picking, while deferred lots were temporarily stored in the 48-50° F. room until the time designated for storage at lower temperature. The methods used for temperature and humidity control have been described in a previous publication (16). Preliminary inspections were made within the storage rooms and the final condition of the fruit was determined early in March each season.

METHODS OF SAMPLING AND ANALYSES

Samples of fruit were analyzed for non-colloidal, colloidal and total nitrogen; for reducing, non-reducing and total sugars; and for alcohol insoluble residue. The first year the sampling began on August 1, while the fruit was green, and continued bi-weekly until the commercial picking date, and thereafter bi-weekly from various storage lots. During the second and third years the chemical investigations were confined to samples of fruit taken on the date of picking and after the normal storage period. The variety Grimes Golden was used for most of the work, but many analyses were also made on Jonathan. The samples consisted of duplicate 100-gram lots of thinly sliced tissue from the centers of 20 apples of medium size selected from the tree and storage lots. These were preserved in alcohol. Extraction was by the decantation method. Non-colloidal and colloidal nitrogen was determined from the extract and alcohol insoluble residue, respectively by the official Kjeldahl method. Reducing and non-reducing sugars were determined from the cleared extract by the Munson-Walker-Bertrand volumetric method, and alcohol insoluble residue by weighing the dried residue remaining after extracting.

RESULTS OF STORAGE TESTS

The storage results for the three seasons for both Grimes and Jonathan are given in table 1. The data for the first season indicate that the nitrogen treatments increased the susceptibility of the fruit to soggy breakdown. However, in the following seasons (1930 and 1931), which were unusually dry, not enough breakdown occurred in any of the storage treatments to give conclusive results. The data suggest that dry weather was the controlling factor in 1930 and 1931, rather than nitrate fertilization. The investigations on Jonathan breakdown of Overley and Overholser (14) and of Palmer (15) indicate clearly seasonal differences in susceptibility, and the former investigators (l. c.) pointed out that nitrogen fertilization increased susceptibility in seasons when the disease was common. Recent studies by Plagge and Maney (16) also lead to similar conclusions.

RESULTS OF ANALYSES

The results of the analyses of the fruit will be considered under separate headings, i. e., nitrogen, sugar and insoluble residue. In each case, the data have been summarized and are representative of the general results obtained. For detailed reports of individual analyses, the reader is referred to the original thesis by the author (17).

NITROGEN

Table 2 gives the nitrogen content of Grimes and Jonathan apples on the picking dates of the three seasons. The data show the results for the nitrated and check treatments of fertilized and check orchards, respectively. The differences between the two treatments in 1929 were slight and fruit from the check trees had approximately the same nitrogn content (including both non-colloidal and colloidal forms), as that from nitrated trees. However, in 1930 and 1931 fruit from fertilized trees of both Grimes and Jonathan was consistently higher in total nitrogen. The differences were principally in non-colloidal nitrogen.

TABLE 1. *Effect of nitrate fertilizer and storage treatment on soggy breakdown in Grimes and Jonathan. Percentage of breakdown at 30–31° F.*

Grimes

Season 1929–30

Treatment before storing	0–0–0*	0–5–0	5–5–0	5–5–5–5	5–5–10
Stored directly	2.5	4.6	14.6	4.7	5.0
Deferred 10 days	......	28.6	21.2	24.6	......
Deferred 20 days	11.1	29.2	53.8	41.0	43.7

Season 1930–31

Treatment before storing	0–0–0–0*	0–5–0–0	5–5–0–0–5	5–5–5–5	5–5–10–10–10
Stored directly	1.4	3.9	2.2	2.4	8.8
Deferred 10 days	17.2	0.0	1.3	0.3	0.0
Deferred 20 days	7.5	0.0	7.3	0.2	0.0

Season 1931–32

Treatment before storing	0–0–0–0–0*	0–5–0–0–0	5–5–0–0–5	5–5–5–5–5	5–5–10–10–10
Stored directly	......	......	0.0	0.0	....
Deferred 10 days	......	......	......	...	....
Deferred 20 days	0.00	2.96	1.38	16.3	0.00

Jonathan

Season 1929–30

Treatment before storing	0–0–0	0–5–0	5–5–0	5–5–5	5–5–10
Stored directly	9.8	47.7	77.5	76.2	66.2
Deferred 10 days	0.0	19.6	34.8	28.2	14.3
Deferred 20 days	0.0	10.7	2.8	1.4	1.9

Season 1930–31

Treatment before storing	0–0–0–0	0–5–0–0	5–5–0–0	5–5–5–5	5–5–10–10
Stored directly	0.0	0.3	1.1	8.7	4.6
Deferred 10 days	0.0	0.0	0.0	0.0	0.0
Deferred 20 days	0.0	0.0	0.0	0.0	0.0

Season 1931–32

Treatment before storing	0–0–0–0–0	0–5–0–0–0	5–5–0–0–5	5–5–5–5–5	5–5–10–10–10
Stored directly	0.00	0.00	0.00	0.00	0.00
Deferred 10 days	1.83	3.24	6.83	12.20	5.32
Deferred 20 days	0.00	0.00	0.00	0.00	0.00

*Numerals 0, 5 and 10 indicate the amount of nitrate fertilizer in pounds per tree applied during different years. Thus: 0–5–0 in-dicates no application in 1927, 5 pounds in 1928 and no application in 1929; while 5–5–10–10 indicates an application of 5 pounds per tree each year for 1927 and 1928, and 10 pounds per tree each for the years 1929 and 1930.

TABLE 2. *Nitrogen content of apples in different seasons in samples analyzed on the commercial picking date.*

Grimes

Year	Non-colloidal N. (mg.)		Colloidal N. (mg.)		Total N. (mg.)	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
1929	16.14	19.52	24.32	24.42	40.46	43.94
1930	14.53	23.00	19.93	26.72	34.46	49.72
1931	8.79	22.54	22.62	21.14	31.41	43.68

Jonathan

Year	Non-colloidal N. (mg.)		Colloidal N. (mg.)		Total N. (mg.)	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
1929	18.30	15.97	22.45	21.17	40.75	37.14
1930	11.05	18.05	20.01	23.82	31.06	41.87
1931	8.64	19.76	14.92	17.32	23.56	37.08

TABLE 3. *Effect of varying the application of nitrate of soda on the nitrogen content of the fruit. Season 1929-30. Storage temperature 30°-31° F. Fruit stored immediately.*

Grimes

Date of sampling	Non-colloidal nitrogen (mg.)			
	Check	Plus nitrogen		
	0-0-0	0-5-0	5-5-0	5-5-10
Sept. 26*	16.136	10.805	15.848	19.522
Jan. 6	13.080	4.660	6.160	14.355

Date of sampling	Colloidal nitrogen (mg.)			
	Check	Plus nitrogen		
	0-0-0	0-5-0	5-5-0	5-5-10
Sept. 26*	24.322	21.113	20.841	24.420
Jan. 6	27.733	21.446	23.295	28.340

Date of sampling	Total nitrogen (mg.)			
	Check	Plus nitrogen		
	0-0-0	0-5-0	5-5-0	5-5-10
Sept. 26*	40.460	31.920	36.699	43.940
Jan. 6	40.810	26.110	29.466	42.690

*Picking date.

The data in table 3 show the effect of the various nitrate treatments within the fertilized orchard on the nitrogen content of Grimes apples during the seasons 1929-30. Lower nitrogen values for the low nitrate treatments (0-5-0 and 5-5-0) were consistent for both the non-colloidal and colloidal forms, at the end as well as at the beginning of the storage period. Non-colloidal nitrogen decreased considerably in fruit from the low nitrogen treatments of the fertilized orchard, while colloidal nitrogen remained the same. There was no marked decrease in non-colloidal nitrogen during storage in either of the samples from the check treatment of the unfertilized orchard or from the high nitrogen treatment. The similarity in nitrogen content between samples from these two treatments noted on the picking date was again apparent after storage.

The nitrogen content of Grimes fruit in 1930, as affected by various nitrate treatments, is shown in table 4. In this year nitrate fertilization consistenly increased the total nitrogen content of the fruit and, in most instances, both non-colloidal and colloidal nitrogen content. The most marked increases of nitrated over unnitrated samples were in the non-colloidal form. The low nitrate treatments of the fertilized orchard were approximately of the same total nitrogen content as the treatments of the unfertilized orchard, while the normal (5-5-5-5) and plus (5-5-10-10)

TABLE 4. *Effect of varying the application of nitrate of soda on the nitrogen content of the fruit. Season 1930-31. Fruit stored immediately in cold storage*

Grimes

Date of sampling	Stor. temp. F.	Non-colloidal nitrogen (mg.)				
			Plus nitrogen			
		Check	0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	14.535	9.715	10.040	18.180	23.000
April 1	30°-31°	11.945	5.185	4.509	9.694	26.500
April 1	35°-36°	12.705	3.908	4.734	9.543	15.900

Date of sampling	Stor. temp. F.	Colloidal nitrogen (mg.)				
			Plus nitrogen			
		Check	0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	19.926	21.007	20.586	23.937	26.717
April 1	30°-31°	29.021	22.257	21.063	26.489	31.190
April 1	35°-36°	24.051	20.054	23.109	28.749	32.126

Date of sampling	Stor. temp. F.	Total nitrogen (mg.)				
			Plus nitrogen			
		Check	0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	34.461	30.722	30.626	42.117	49.720
April 1	30°-31°	40.966	27.442	25.752	36.183	57.690
April 1	35°-36°	36.756	23.962	27.843	38.292	48.026

*Picking date.

nitrate treatments yielded fruit considerably higher in nitrogen content. Non-colloidal nitrogen decreased during storage under the low and normal nitrate treatments at both storage temperatures 30-31° F. and 35-36° F.

A study of the nitrogen content of apples during the storage period was made. The nitrogen content of Grimes was determined approximately at monthly intervals in 1929-30. The results obtained for storage at 48-50° F. are given in tables 5 and 6, respectively. These data show that non-colloidal nitrogen decreased rapidly in untreated fruit during the first 20 days of storage at 48-50° F. Fruit from the check treatments decreased approximately 60 per cent, while that from the nitrate treatment decreased only 18 per cent in non-colloidal nitrogen. In table 6 it will be noted that non-colloidal nitrogen was higher in fruit from the fertilized trees throughout the storage period, because of the more rapid loss in the apples from unfertilized trees during the first three weeks of storage at 48-50° F. This point is of interest in connection with the nitrogen analyses of peaches reported by Nitghtingale *et al* (13), who found that unfertilized fruits had a higher percentage of nitrogen present in the protein-like form, whereas fruit from nitrated trees which had more nitrogen present had most of it in the simpler amino acid form. On the other hand, Thomas (18)

TABLE 5. *Nitrogen content of Grimes. Season 1929-30. Fruit stored immediately at 48°-50° F.*

Date of sampling	Non-colloidal N. mg.		Colloidal N. mg.		Total N. mg.	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Sept. 26	16.136	19.522	24.322	24.420	40.458	43.942
Oct. 17	6.411	15,920	21.016	28.609	27.427	44.529
Nov. 6	7.060	11.670	20.263	25.777	27.323	37.447
Dec. 1	3.890	13.975	25.590	30.592	29.480	44.567
Jan. 6	6.689	11.650	24.857	30.513	31.546	42.163

TABLE 6. *Nitrogen content of Grimes. Season 1929-30. Storage date deferred 20 days. Storage temperature 30°-31° F.*

Date of sampling	Non-colloidal N. mg.		Colliodal N. mg.		Total N. mg.	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Sept. 26*	16.136	19.522	24.322	24.420	40.458	43.942
Oct. 17**	6.411	15.920	21.016	28.609	27.427	44.529
Nov. 6	8.644	9.941	21.983	24.704	30.627	34.645
Dec. 1	6.339	11.800	24.942	28.155	31.281	39.955
Jan. 6	8.493	15.032	23.776	28.843	32.269	43.875
March 15	8.268	18.940	25.026	27.291	33.266	46.231

*Picking date.
**Storage date.

found a close parallelism between total water soluble nitrogen and free amino nitrogen throughout the cycle in all tissues of the apple, and suggested the possibility that amino acids are the chief fractions influencing growth and vigor of the tree.

Therefore, it seems that the non-colloidal nitrogen fraction, as determined in this investigation, may be the important factor in regulating the metabolism of the apple, and thus offer an explanation for greater susceptibility to soggy breakdown in apples from fertilized trees. The non-colloidal nitrogen content of fertilized fruits was over 100 per cent higher in Grimes after three weeks of deferred storage and continued thus after five and one-half months in storage at 30-31° F.

This investigation as carried out offered an opportunity to observe whether there is a residual effect in the nitrogen content of the tree resulting from nitrate fertilization, and whether this is reflected in the nitrogen content of the fruit. In table 3, the data for Grimes in 1929 show that there was a greater proportion of non-colloidal and total nitrogen under treatment 5-5-0 as compared with treatment 0-5-0, which indicates that the 1927 application of nitrate increased the nitrogen content of the fruit in the 1929 year. However, the results for 1930 (table 4) do not indicate that a residual nitrogen content, if present in the tree, influenced the nitrogen content of the fruit. Treatments 0-5-0-0 and 5-5-0-0 were about the same in nitrogen content; in other words, the 1927 nitrate application did not appear to affect the nitrogen content of the fruit in 1930. It appears, therefore, that nitrate fertilizers increased the nitrogen content of the fruit the first and second years after applying, but not in the third year. The principal differences were in the non-colloidal form.

SUGAR

The sugar content of Grimes apples before commercial harvest was studied in 1929. The percentage of sugar for both the check and high nitrogen treatments for samples picked at intervals during ripening is given in table 7. It will be noted that reducing and total sugars were consistently lower in the high nitrogen samples, with the exception of the first sample taken on August 1. The same was true for non-reducing sugars except for one analysis.

TABLE 7. *Percentage of sugar in Grimes. Season 1929–30. Early picked fruit*

Date of sampling	Reducing sugars		Non-reducing sugars		Total sugars	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Aug. 1	4.74	5.11	1.61	1.70	6.34	6.82
Aug. 15	5.46	4.84	3.13	1.45	8.59	6.28
Sept. 5	5.86	5.01	2.42	2.89	8.29	7.90
Sept. 16	5.63	5.08	3.73	2.83	9.36	7.91
Sept. 26*	5.79	5.77	4.81	4.03	10.61	9.80

*Commercial picking.

The results of analyses taken on fruit stored immediately at 30-31° F. in 1929 are shown in table 8. In this case total and non-reducing sugars

are usually higher in the fruit from the check trees. The results give a picture of the sugar content throughout the storage period at 30-31° F.

TABLE 8. *Percentage of sugar in Grimes. Season 1929-30. Fruit stored immediately at 30°-31° F.*

Date of sampling	Reducing sugars		Non-reducing sugars		Total sugars	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Sept. 26	5.79	5.77	4.81	4.03	10.61	9.80
Oct. 17	6.06	6.05	4.89	4.63	10.95	10.67
Nov. 6	6.15	6.16	5.10	4.56	11.25	10.72
Dec. 1	6.14	6.52	4.97	4.63	11.11	11.14
Jan. 6	6.63	6.59	4.88	4.88	11.51	11.47
March 15	7.24	8.26	4.06	2.56	11.30	10.82

The sugar content of Grimes apples throughout the storage period at 48-50° F. is shown in table 9. In this case, again a slightly higher total sugar level in the fruit from the unfertilized trees was maintained throughout the storage period. Sucrose content also was consistently higher in the check fruit, while reducing sugar showed an increase in the nitrated fruit during the latter part of the season.

TABLE 9. *Percentage of sugar in Grimes. Season 1929-30. Fruit stored immediately at 48°-50° F.*

Date of sampling	Reducing sugars		Non-reducing sugars		Total sugars	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Sept. 26	5.79	5.77	4.81	4.03	10.61	9.80
Oct. 17	6.14	5.95	5.76	5.61	11.90	11.56
Nov. 6	6.64	6.73	5.43	4.90	12.07	11.63
Dec. 1	6.94	7.06	5.21	4.73	12.15	11.79
Jan. 6	7.38	8.29	4.32	3.70	11.70	11.47

The data in table 10 show that there was a higher sugar level in check fruit during the second year of the investigation. These data indicate that total sugars and sucrose were slightly higher in check fruit, as well as in the minus nitrogen fruit from the fertilized orchard. Reducing sugars fluctuated more than non-reducing sugar; the latter decreased, while the former increased during storage.

It has been pointed out that 1930 and 1931 were below normal in rainfall. The year 1929 was almost average in the amount of rainfall, while 1928 was excessively wet. Analyses of both Grimes and Jonathan apples on the picking date were made in 1931, so that a comparison of the sugar content for the three years 1929, 1930 and 1931 is possible. The data in table 11 show these results. Total sugars in orchard A Grimes in the two dry years (1930 and 1931) were slightly higher, but in the case of orchard B fruit, the results were less consistent. Likewise, with Jonathan, total sugars were not consistently higher in 1930 and 1931. Non-reducing

TABLE 10. *Effect of various nitrate applications on percentage of sugar in Grimes. Season 1930-31. Fruit stored immediately*

Date of sampling	Storage temp. F.	Percentage of reducing sugars				
		Check	plus nitrogen			
			0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	6.29	7.11	7.05	7.36	7.01
April 1	30°-31°	8.48	8.32	8.28	8.42	8.16
April 1	35°-36°	8.61	8.64	7.84	8.05	8.52

Date of sampling	Storage temp. F.	Percentage of non-reducing sugars				
		Check	plus nitrogen			
			0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	4.08	3.84	3.91	2.94	3.29
April 1	30°-31°	3.03	3.47	2.75	3.39	2.92
April 1	35°-36°	3.24	2.84	3.01	2.86	1.80

Date of sampling	Storage temp. F.	Percentage of total sugars				
		Check	plus nitrogen			
			0-5-0-0	5-5-0-0	5-5-5-5	5-5-10-10
Sept. 24	*	10.37	10.95	10.96	10.30	10.30
April 1	30°-31°	11.52	11.79	11.02	11.81	11.08
April 1	35°-36°	11.85	11.48	10.86	10.91	10.32

*Picking date.

sugars were correspondingly lower and reducing sugars higher in Grimes fruit in the two dry years, but a similar relationship did not hold for Jonathan. We may conclude, therefore, that dry weather in 1930 and 1931 did not increase the sugar content of apples over that of 1929.

The data on sugar analyses in the above tables are considered to be evidence that nitrate fertilization of apple trees may result in slightly lowering the total sugar and non-reducing sugar content of the apple. Still other data obtained with Grimes apples under other storage treatment and further data on Jonathan apples might be cited, but these would only emphasize what has already been indicated in the above tables. The results are in harmony with those reported by Hopkins and Gourley (9) and Hopkins and Greve (10), who in a similar investigation found small but consistent differences in the soluble carbohydrates between apples from nitrated and unnitrated trees. The results herein reported show that the most marked changes during storage were in sucrose content. In a few instances sucrose reached a very low level in fruit from the nitrated trees. This was especially true in the case of fruit stored at the higher temperature (48-50° F.). During the storage period reducing-

TABLE 11. *Percentage of sugar in apples in different seasons. Analyses on the commercial picking date*

Grimes

Year	Reducing sugars		Non-reducing sugars		Total sugars	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
1929	5.79	5.77	4.81	4.03	10.61	9.80
1930	6.29	7.01	4.08	3.29	10.37	10.30
1931	6.42	6.37	4.50	3.75	10.92	10.12

Jonathan

Year	Reducing sugars		Non-reducing sugars		Total sugars	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
1929	7.57	6.12	3.51	4.14	11.08	10.27
1930	8.16	7.62	3.05	2.38	11.21	9.99
1931	7.24	7.67	3.70	4.04	10.94	11.71

sugar values indicated more stability than sucrose values, and this result is in accordance with those of Evans (5), who found reducing sugar content in apples to fluctuate within a narrower margin than sucrose and total sugar contents. These results suggest that reducing sugars are supplied to the apple (by hydrolysis of sucrose) about as fast as they are being oxidized.

THE RATIO OF SUGAR TO NITROGEN AS AN INDICATION OF RESISTANCE TO SOGGY BREAKDOWN

If sugar content may be taken as a measure of stored reserve material in the apple, and nitrogen as a measure of catabolic activity, then the proportion of sugar to nitrogen may be indicative of resistance to breakdown or the storage capacity of the fruit. Haynes and Archbold (8) have suggested a relation between the ratio of sugar to nitrogen content of the apple and the length of storage life. The differences noted in the resistance to soggy breakdown between apples from unfertilized and fertilized trees, and between the fruit stored in different seasons, suggest the comparison of sugar to nitrogen ratios in comparable samples. Such ratios measured in mg. of reducing sugar and sucrose per mg. of nitrogen, on samples taken on the harvest dates for the three seasons, are depicted in figures 1, 2, 3 and 4.

Considering the ratios of reducing sugar to the nitrogen fractions and to total nitrogen (Figs. 1 and 2), it will be noted that these ratios were all higher for the two dry years (1930 and 1931) as compared with 1929. The situation held for both varieties under both treatments (fertilized and unfertilized orchards) and suggests a reason for the consistent higher resistance to soggy breakdown in Grimes and Jonathan apples in 1930 and 1931. It appears from these data that the reducing sugar/non-colloidal
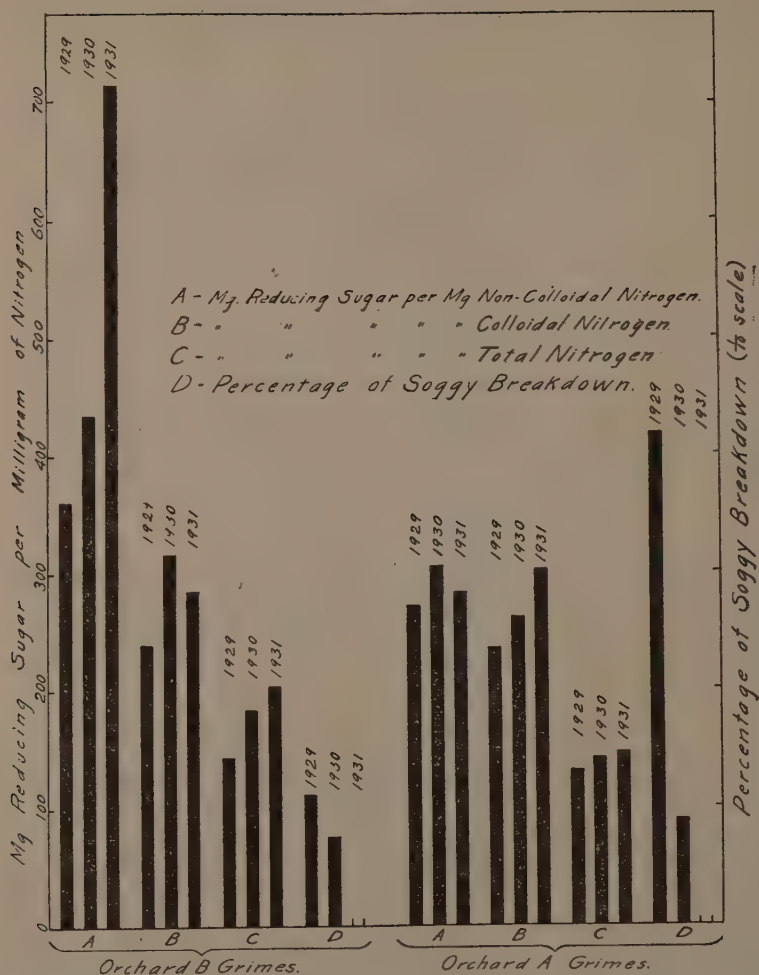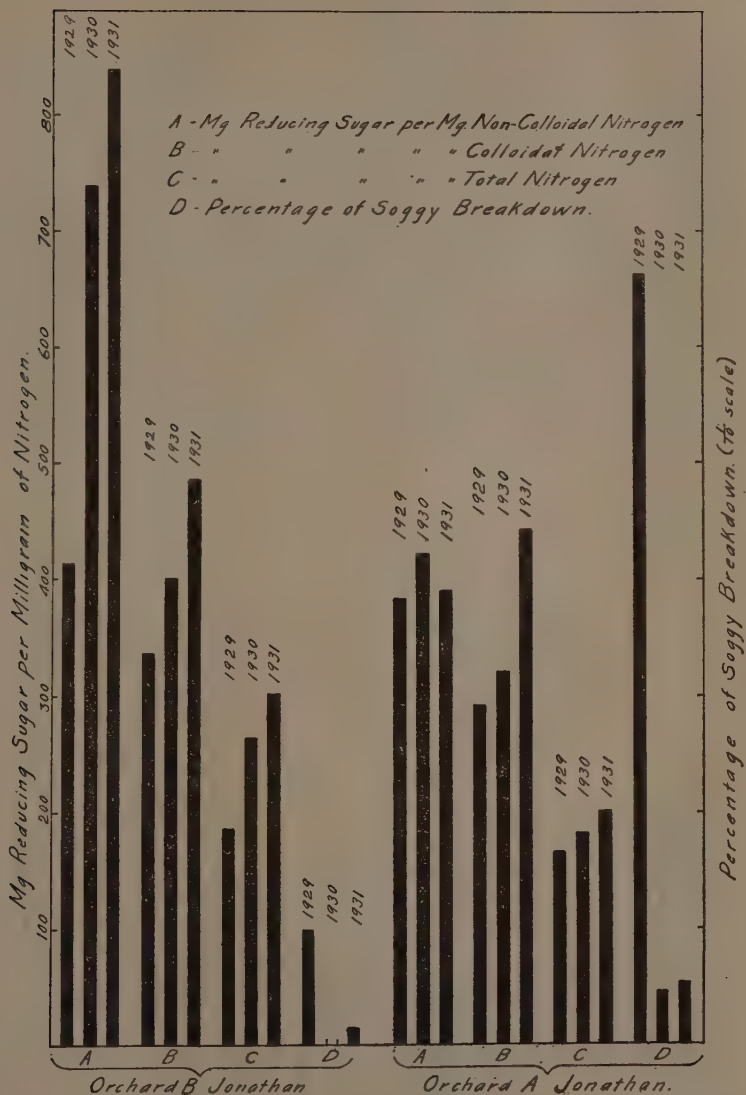
Fig. 1. Diagram showing correlations between the reducing sugar to nitrogen ratios and soggy breakdown in Grimes apples.

nitrogen ratio is a better indicator of resistance to soggy breakdown than the reducing sugar/colloidal nitrogen ratio, since in Grimes in 1929 (Fig. 1) the two values of the latter ratio for the two treatments were more nearly the same. Moreover, the differences in reducing sugar to colloidal nitrogen ratios between samples from the two orchards in 1930 and 1931 were less marked than the differences in reducing sugar/non-colloidal ratios.

It will be noted (Figs. 1 and 2) that reducing sugar/non-colloidal nitrogen values were consistently higher in the fruit from check trees

Fig. 2. Diagram showing correlations between the reducing sugar to nitrogen ratios and soggy breakdown in Jonathan apples.

(orchard B fruit) than in the fruit from fertilized trees (orchard A fruit). This status held between comparisons in both varieties and in each of the three years of study. These higher reducing sugar/non-colloidal nitrogen

ratios in orchard B fruit again suggest a reason for greater resistance to
soggy breakdown, which is in agreement with storage results when
breakdown differences were apparent in 1929. Although differences in
soggy breakdown were not apparent in 1930 and 1931, it is suggested that
some other undetermined factor or factors associated with warm, dry
weather were operative in making the fruit more resistant to this dis-
order. Among such may be listed the alcohol insoluble residue/nitrogen
ratio, the total sugar plus organic acids/nitrogen ratio and others.

The relationship between the sucrose/nitrogen ratio on the picking
date and the resistance to soggy breakdown in storage is of interest in this
connection. Haynes and Archbold (l. c.) have pointed out that the supply
of sucrose may be a limiting factor in the storage of apples and that the
sucrose/nitrogen ratio may be indicative of storage behavior. A graphic
representation of the sucrose/non-colloidal nitrogen, sucrose/colloidal
nitrogn and sucrose/total nitrogen ratios for Grimes and Jonathan fruit
from the two orchards A and B is shown in figs. 3 and 4, respectively. It
will be noted that there were no consistent differences between the
sucrose/nitrogen ratios in samples of fruit of the different years. That is,
the ratios of sucrose to the various forms of nitrogen for the two years
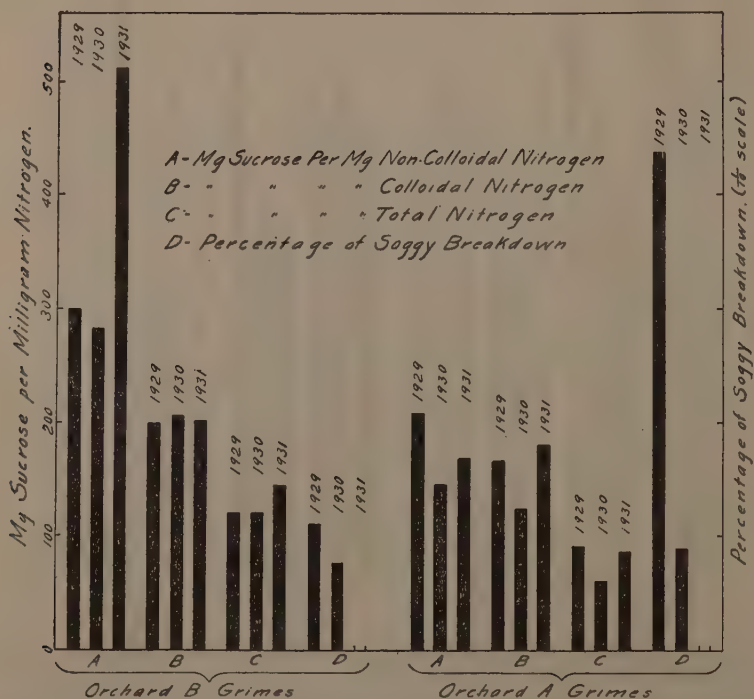
Fig. 3. Diagram showing correlations between the sucrose to nitrogen ratios and
soggy breakdown in Grimes apples.

1930 and 1931, when the fruit was highly resistant to soggy breakdown, were not consistently higher than the corresponding ratios in 1929, when the fruit was susceptible to soggy breakdown.

In a similar manner, in the comparison between the sucrose/nitrogen ratios of the fruit from the two orchards in the same years there also is no consistent relationship between these ratios and the percentage of soggy breakdown. The sucrose/non-colloidal nitrogen, sucrose/colloidal nitrogen and sucrose/total nitrogen ratios, as determined on the date of picking, therefore do not appear to have been indicative of the relative susceptibility of these apples to soggy breakdown. An explanation for the inconsistency of the sucrose/nitrogen ratio as an index of resistance to soggy breakdown in apples probably lies in the variability in sucrose
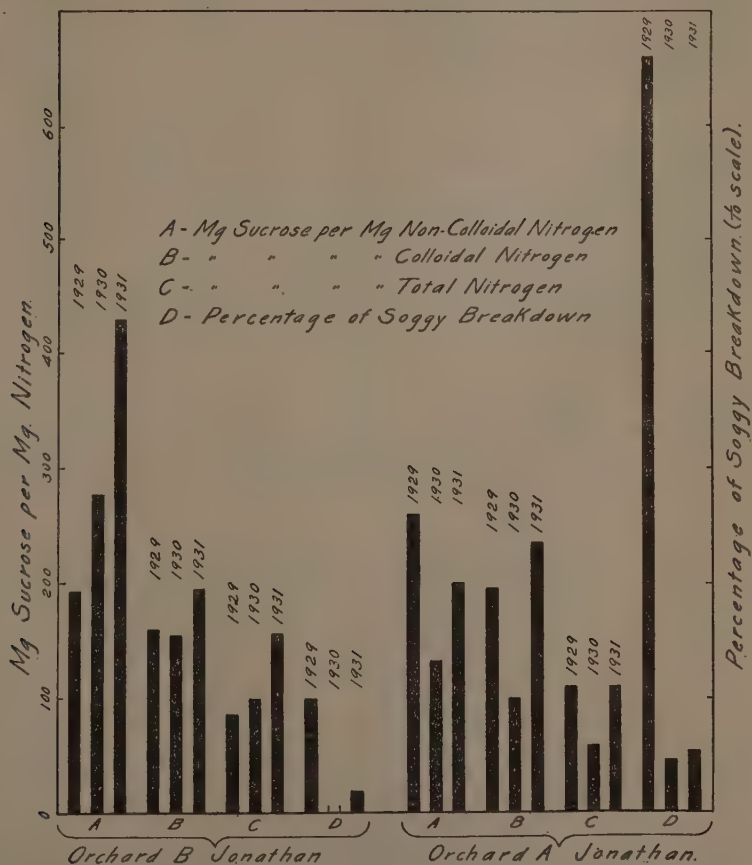
Fig. 4. Diagram showing correlations between the sucrose to nitrogen ratios and soggy breakdown in Jonathan apples.

content of individual apples because of a rapidly changing starch/sucrose ratio at picking time and during the latter part of the ripening period. Starch content decreases in fruit on the tree during this period and there is a corresponding increase in sucrose content. Reducing sugars change less than sucrose. The data in table 7 show that orchard A Grimes in 1929 increased over 42 per cent in sucrose content during the last 10 days on the tree, while reducing sugars increased only 13.5 per cent during the same interval. It appears, therefore, that sucrose content is more variable than reducing sugar in apples while ripening and this may be an explanation of the variability noted in the sucrose/nitrogen ratios.

ALCOHOL INSOLUBLE RESIDUE

Alcohol insoluble residue material was determined incidental to obtaining the analyses of colloidal nitrogen. It consisted of the tissue remaining after extraction with 80 per cent alcohol and included the insoluble materials starch, pectin, cellulose and other substances. Residues were determined on duplicate samples and close agreement was obtained in all cases.

The changes in residue values for Grimes fruit stored in 1929 are shown in tables 12 and 13. With samples stored immediately (table 12)

TABLE 12. *Alcohol insoluble residue of Grimes apples during storage. Fruit stored immediately. Season 1929–30. Grams per 100 gm. of fresh weight*

Date of sampling	30°–31° F.		35°–36° F.		48°–50° F.	
	Check	Plus N.	Check	Plus N.	Check	Plus N.
Sept. 26	4.120	4.534	4.120	4.534	4.120	4.534
Oct. 17	3.615	3.744	3.364	3.834	3.320	3.134
Nov. 6	3.148	3.278	3.053	3.275	2.775	2.707
Dec. 1	2.829	3.008	2.866	2.805	2.636	2.625
Jan. 6	2.754	2.723	2.691	2.624	2.585	2.571
March 15	2.670	2.646	2.695	2.679	....	....

TABLE 13. *Alcohol insoluble residue of Grimes apples during storage. Fruit deferred at 48°–50° F. then stored in cold storage. Season 1929–30. Grams per 100 gm. of fresh weight*

Date of sampling	30°–31° F.		35°–36° F.	
	Check	Plus N.	Check	Plus N.
Sept. 26	4.120	4.534	4.120	4.534
Oct. 17*	3.615	3.744	3.364	3.834
Nov. 6	2.937	2.762	2.789	2.723
Dec. 1	2.751	2.607	2.774	2.570
Jan. 6	2.622	2.548	2.683	2.547
March 15	2.603	2.519	2.641	2.564

*Storage date.

the residues of plus nitrogen fruit remained higher with the 30-31° F. treatment until December 1; with the 35-36° F. treatment until November 6; with the 48-50° F. treatment only until a short time after storing, which was before the second analysis on October 17. With samples given deferred storage treatment (table 13) the residues of plus nitrogen fruit remained higher with the 30-31° F. and 35-36° F. treatments only until the date of storage (October 17).

It has been pointed out that sucrose content in the plus nitrogen Grimes in 1929 increased over three times as fast the last 10 days on the tree as check Grimes. It is suggested, therefore, that the higher residue of plus nitrogen fruit on the date of picking was due chiefly to the presence of more starch, probably as a consequence of slightly less maturity. Archbold (3) found the loss in alcohol insoluble residue on the tree in August to be due almost entirely to hydrolysis of starch. It seems reasonable to conclude, therefore, that after starch hydrolysis was complete in storage the subsequent higher residue content in the unfertilized fruit was the result of greater cell wall development, coincident with a higher degree of differentiation of wall tissues in the growth of the fruit. Since a greater quantity of cell wall material indicates more storage reserves in the form of pectic constituents and alcohol insoluble acid hydrolizable materials, this offers an explanation for premature breakdown in apples from nitrogen fertilized trees. It has been shown that more soggy breakdown occurred in fertilized fruit in 1929 than in check fruit, but that little difference in breakdown occurred between these treatments in 1930 and 1931. However, in seasons when fruit growth and ripening take place under normal seasonal conditions, it appears that the alcohol insoluble residue of apples can be taken as one measure of storage capacity.

SUMMARY

1.   Sodium nitrate was applied, in various quantities and in different years in a high producing orchard, to Grimes and Jonathan trees. Samples of fruit from the nitrate treatments and from an adjacent untreated orchard were analyzed for nitrogen and sugar content at various intervals before picking and after storing throughout two seasons.

2.   Sodium nitrate fertilizer appears to have greatly increased the susceptibility of Grimes and Jonathan apples to soggy breakdown in 1929, a year having about a normal growing season. In 1930 and 1931 little soggy breakdown occurred and no significant differences in the susceptibility of fruit taken from the plus nitrogen trees and minus nitrogen trees in the same orchard, or from the untreated trees in another orchard, were apparent.

3.   Within the same orchard, nitrate applications consistently increased the nitrogen content of the fruit in each of the three years of this investigation. These increases were principally in the non-colloidal fraction. Within different orchards, the nitrated orchard produced apples higher in nitrogen content during the two dry years (1930 and 1931), but in the wet year (1929) the differences between the fruit from the two orchards were not significant. During the last two seasons, when nitrogen content varied significantly, little or no breakdown appeared in the

fruit. Therefore, there was no correlation between the nitrogen content of the fruit when it was picked and soggy breakdown susceptibility.

4. Non-colloidal nitrogen decreased in apples in storage. This decrease was more pronounced at the higher temperature employed (48-50° F.) than at cold storage temperatures. The results indicate that non-colloidal nitrogen in apples was modified more by storage temperature than the colloidal form. Under deferred storage at 48-50° F. there was a greater and more rapid loss of non-colloidal nitrogen in fruit from the unfertilized trees than in fruit from the nitrated trees. A higher non-colloidal nitrogen level in the plus nitrogen samples persisted throughout five and one-half months in storage.

5. Nitrate fertilizers appeared to increase the nitrogen content of the fruit harvested the same year of application, as well as that of fruit harvested the second year after application. However, fruit from trees which had not received nitrate fertilizer for three years showed no increased nitrogen content as a result of the treatment. The increased nitrogen content did not make the fruit more susceptible to soggy breakdown.

6. The results on sugar analyses, although not always consistent, showed in general that nitrate fertilizer slightly reduced the sugar content of the fruit. The data indicate that the sugar content of the fruit, when it is placed in storage could not be taken as a measure of breakdown susceptibility. The most marked changes during storage were in sucrose content; in a few instances sucrose reached a very low level in fruit from the nitrated trees. This was especially true in the case of fruit stored at the higher temperatures (48-50° F.). During the storage period reducing-sugar values indicated more stability than sucrose values.

7. Ratios of sugar to nitrogen indicated higher values of reducing sugar to the nitrogen fractions for the two dry years (1930 and 1931) as compared with 1929. This situation was consistent for both Grimes and Jonathan varieties, and for fruit from either the nitrated or check orchard. This result suggests a reason for the consistently higher resistance to soggy breakdown in 1930 and 1931, when little occurred. The reducing sugar/non-colloidal nitrogen ratio appeared to be a better indicator of resistance to soggy breakdown than the reducing sugar/colloidal nitrogen ratio.

8. Reducing sugar/non-colloidal nitrogen values were consistently higher in fruit from the unfertilized orchard than that from the fertilized orchard. The latter situation held for both varieties and for each of the three seasons and suggests a reason for greater resistance to soggy breakdown in fruit from the check orchard.

9. Ratios of sucrose to either of the nitrogen fractions determined, or to total nitrogen, taken on the picking date could not be correlated with susceptibility to soggy breakdown.

10. Alcohol insoluble residues from fertilized samples remained higher than those from check samples only during the forepart of the storage season. During the latter part of the storage period, after complete starch hydrolysis, the residues from the check samples were greater. The

exchange from higher residue values to lower, and vice versa, took place early or late, depending upon storage temperature. The consistently higher alcohol insoluble residue (after complete starch hydrolysis) in fruit from check trees suggests more cell wall differentiation in this fruit, and suggests one other reason for better storage capacity.

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THE RELATION OF THE SIZE OF THE INFECTIVE DOSE TO NUMBER OF OÖCYSTS ELIMINATED, DURATION OF INFECTION, AND IMMUNITY IN EIMERIA MIYAIRII OHIRA INFECTIONS IN THE WHITE RAT[1]

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To the author's knowledge, previous investigators have not determined (1) the number of oöcysts eliminated following the feeding of various sized doses of viable sporulated oöcysts of a coccidium of the genus Eimeria, (2) whether the length of time during which oöcysts were passed in the feces varied with the size of the infective dose, (3) the relative amount of immunity produced by small infective doses of varying size, and the minimum number of sporulated oöcysts required to produce total immunity and (4) the pathological effects of different sized doses, such as effects on the mean daily weights of the hosts. The present study was designed to make such determinations.

Rats used in these experiments were Wistar A rats (excepting possibly one litter used in the series infected with one oöcyst per rat), and were offspring of closely related parents. By using rats of such parentage, all experimental animals had a similar genetical constitution and consequently differences in yields due to hereditary differences in susceptibility of the host were avoided as much as possible. It was noted that even then there appeared litters which as a group out-yielded other litters which had received a duplicate treatment. From the time of birth until approximately four weeks later, each litter of young rats was kept in a breeding cage, the bottom of which was covered with a carpet of dry shavings.

The young rats, after being weaned, were fed a modification of the Steenbock growth ration, green food at intervals of three or four days, and milk and water *ad libitum*. The ration used was as follows:

Yellow corn meal	76.0 lbs.	Ground alfalfa	2.0 lbs.
Linseed oilmeal	16.0 "	NaCl	0.5 "
Commercial casein	5.0 "	$CaCo_3$	0.5 "
	Dried buttermilk	12.0 lbs.	

At the age of five weeks, the rats were put on experiment. At this time each animal was placed individually into a specially made cage of

three mesh hardware cloth fitted over a 9″ x 12″ aluminum or enamel pan. The rats were weighed daily after being put on the experiment. The diet, thereafter, consisted of only the grain mixture and water. Previous to the time of the experimental feeding of oöcysts, none of the rats had an infection. Until then, all young growing rats were examined at regular intervals of three or four days, and had there been accidental infection, it would have been detected.

The parasite used in the experiment was a selected strain of *Eimeria miyairii*. This strain was selected and inbred in the following manner. With a micropipette a single sporulated oöcyst was secured from a field under low (100X) power of the microscope. Then by placing the pipette into the mouth of a partially anesthetised rat this oöcyst was administered to its host. The oöcysts which developed from this one were collected in a two per cent solution of potassium dichromate, and from this culture another single oöcyst was isolated and similarly administered to another rat. This process was repeated until the parasite had been inbred for the third generation. It was believed at this time that whatever oöcysts might be selected by chance from this highly inbred strain would be representative of a fairly homogeneous population.

1. NUMBER OF OÖCYSTS PRODUCED BY DIFFERENT SIZED INFECTIVE DOSES

The number of oöcysts eliminated following a series of five infective doses of 1,500 oöcysts each was reported in 1932 by Becker and Hall as falling between 14,100,000 and 169,220,000. No study, however, has been made to determine the yield from a single dose of a known number of sporulated oöcysts. It was felt that information concerning the normal number of oöcysts produced by any known number of parasites would be of value in the study of effects produced during an infection, and also would determine the optimum dose for experimental purposes with the rat as the host. It was with these motives in view that the author has attempted to determine the relationship which exists between the number of sporulated oöcysts fed to an animal and the number of parasites passed in the feces of the infected animal during the period of oöcyst elimination.

In the determination of the yield of oöcysts from a given number of parasites, the infective doses contained the following number of organisms, respectively: one, four, ten, fifty, one hundred, fifteen hundred, fifteen thousand, and fifteen hundred thousand. Of thirty-three attempts to infect rats with a single parasite, only twenty-two trials proved to be successful. From these figures it was concluded that only two-thirds of the number of oöcysts given to any one rat succeeded in completing the sexual phase of their life history in the intestine. This assumption was thereafter respected in the calibration of all cultures used for infective purposes. The small doses of one, four and ten oöcysts were isolated with a micropipette by selecting the individual oöcysts from a field under low power of the microscope. The actual selection consisted of one, six and fifteen oöcysts, respectively.

In giving doses of fifty oöcysts or more, the dilution method was used in measuring the dose. In each of the latter cases, the culture was so calibrated that approximately the desired number of parasites was suspended in one cubic centimeter of liquid.

The rats were etherized and given the cubic centimeter of the suspension containing the oöcysts from a syringe through a catheter. For each group of rats infected with the different sized doses, a number of rats of the same litter were kept for controls.

On the sixth day after the date of infection the pans under the experimental animals were thoroughly cleaned, and to each was added three hundred cubic centimeters disinfectant. The disinfectant used varied from twenty-five hundredths per cent to five-tenths per cent solution of "Kreso," the approximate chemical composition of which is cresol, two and five-tenths per cent; soap (dry), twenty-three per cent; and inert ingredients, seventy-four and five-tenths per cent.

The fecal pellets were allowed to collect in the pans until elimination of oöcysts ceased. The pellets were then broken up by mashing with a miniature stamper consisting of a solid one-inch rubber stopper into one end of which was inserted a glass stirring rod one-fourth inch in diameter and five inches in length. The suspension of fecal matter in the disinfectant was transferred from the collecting pan to a heavy glass container in which it was thoroughly homogenized by an electric mixer. The mixture was diluted with water, the exact dilution depending upon the amount of solids in the feces collected. After the material was again thoroughly agitated, the larger solid particles were removed from a small sample by straining through wire screens. This process was done in a fashion which averted a probable straining out of oöcysts by solid particles accumulated on the filter. Screens through which the material was strained were of two sizes; namely, sixteen and twenty-four mesh, respectively. Following another thorough mixing a small amount of the suspension was immediately transferred to a haemocytometer three-tenths millimeter square and one-tenth millimeter deep. For determining the yield from a single parasite, the counting chamber, or haemocytometer was filled twenty times. For the larger ones, however, only eight or ten counts were made, the number of counts depending on the extent of variation of the first eight. Then from the known number of oöcysts in either eighteen, nine or seven and two-tenths cubic millimeters, the total yield was calculated by multiplying the number of parasites counted by the ratio of the total volume of diluted material to the volume containing the oöcysts counted.

Counts of the oöcysts eliminated by the individual rats[3] on the experiment showed that the greater the infective dose of parasites the greater was the yield of oöcysts, but the ratio was not by any means constant. In table 1 are recorded the mean yields from the different sized infective doses, and the number of oöcysts produced per oöcyst fed in the various infections. When only one oöcyst was fed there was a mean yield of approximated 62,000, while following an infective dose of four viable sporulated oöcysts 2,182,500 oöcysts per oöcyst fed were produced. Why the yield per oöcyst given for four oöcysts should be thirty-five times that of the one oöcyst infection is at present unexplainable. It might be due to increased chances for the union of the microgametes and the macrogametes in the case of the larger infective dose. It can be readily seen that an increase in number of parasites fed would cause the liberation of merozoites in larger quantities, which in turn would develop into more gamete-

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[3]Records of individual rats are printed in the original thesis No. 295 on file in the Iowa State College Library, Ames, Iowa.

producing cells. The larger the number of gametes, the greater their proximity, and consequently the greater the likelihood of the microgametes encountering the macrogametes. This, however, does not explain why the oöcyst production per oöcyst fed should again gradually decrease from 2,084,000 when ten oöcysts were fed to 1,000 when fifteen hundred thousand were fed. The latter may be an immunity phenomenon, or the result of depletion of suitable host cells for colonizing. The nature of the immunity produced and the exact time required for its production are not known, but here again the marked comparative decrease in yield per oöcyst in the case of the higher dosage might perhaps be due to a partial

TABLE 1. *Number of oöcysts produced by different sized infective doses*

Number of animals	Number of oöcysts in infective dose	Mean yield/$(10)^4$	Standard deviation/$(10)^4$	Oöcysts produced per oöcyst fed/$(10)^4$
22	1	6.24 ± .029	.21	6.24
19	4	873 ± 40	260	218.25
15	10	2,084 ± 155	892	208.40
16	50	8,235 ± 502	2,977	164.70
14	100	15,445 ± 1,377	7,360	154.45
11	1,500	28,830 ± 1,323	6,472	19.22
7	15,000	38,254 ± 2,336	9,147	2.61
1	1,500,000	155,000		.10

immunization by the larger numbers of merzoites which prevents the development of a proportionate number of gametocytes, and hence gametes. There remains also the possibility that there is during the infection a temporary depletion of invadable epithelial cells, as suggested above. It seems, however, that if this were the case, the total oöcyst yield would be constant after a maximum was once reached.

2. DURATION OF INFECTION

It was ascertained by daily fecal examination that all rats on the various experiments began to eliminate oöcysts either on the seventh or the eighth day after infection. According to Andrews (1) the time required for the endogenous cycle to become completed is the prepatent period. It will be seen from table 2 that the first appearance of oöcysts was on th seventh day in the majority of cases. Even though oöcysts were not found until the eighth day, all fecal matter eliminated on the seventh day was saved. This precaution was taken for fear there were a few oöcysts eliminated by all rats on the seventh day, but because of random sampling the fecal smears examined did not reveal their presence. In no case was the oöcyst yield at its maximum intensity before the ninth or tenth day after the administering of the viable oöcysts. Table 2 also gives the duration of the period of oöcyst elimination in days. It will there be noted that this period varied from three to five days, inclusive. The data show that the mean patent period varies from 4.2 days following the feeding of small infective doses to 5 days following an infective dose of 1,500,000 oöcysts. The author questions whether there is actually an increase in the

length of the period of oöcyst elimination corresponding to the increase in number of oöcysts fed. The seeming difference may be merely due to the oöcysts being present in larger numbers sooner after elimination begins and for a longer period after the time of maximum yield has been reached. With an increase in the number of oöcysts present, there would be a corresponding increase in the chances for their being seen in the sample of feces examined.

TABLE 2. *Effect of size of dose on duration of infection*

Number of oöcysts in infective dose	Number of rats on experiment	Prepatent period			Patent period			
		7 days	8 days	Mean	3 days	4 days	5 days	Mean
1	22	8	14	7.6	2	12	8	4.2
4	1	9	10	7.5	1	12	6	4.2
10	15	7	8	7.5	1	8	6	4.3
50	16	9	7	7.4		8	8	4.5
100	14	6	8	7.5	1	8	5	4.3
1,500	11	7	4	7.3		3	8	4.7
15,000*	7	5	2	7.2		1	6	4.8
1,500,000*	1	1		7.0			1	5.0

*The data given here apply only to rats which survived throughout the infection.

3. IMMUNIZING EFFECTS OF DIFFERENT SIZED DOSES

It was reported by Becker and Hall (1932) that the feeding of 1,500 viable sporulated oöcysts on each of five consecutive days produced a total immunity. It was realized that five feedings of this dose were more than were required for the production of total immunity. It was suggested by Johnson in 1927 (5) and again by Tyzzer in 1929 (6) that a light infection prevented a heavy subsequent reinfection, but no quantitative study of immunity produced by small infective doses had been made.

To determine whether the rats which had been infected with a given number of oöcysts were either partially or entirely immunized, the previously infected experimental animals together with the controls were given fifteen hundred oöcysts on each of five consecutive days, as it was known that this dose would produce total immunity in the control animals. To avert physiological interference between the two infections the attempts at reinfection were not made until the cessation of elimination of oöcysts produced by the first infection. The same treatment was given to rats during the second infection as during the first, and the previously discussed technique employed in making determinations of oöcyst yields was again used. The yield of oöcysts from previously infected rats compared with that of the controls was taken as an index to the amount of immunity produced by the various sized infective doses.

Efforts to determine the minimum dose which would cause an appreciable loss in susceptibility of the host revealed that an infection as small

as that produced by four oöcysts produces a degree of immunity worthy of note. The yields from the multiple infections following a single infection of 4 oöcysts ranged from 600 x $(10)^4$ to 2,292 x $(10)^4$, while the yield from the controls for that same group ranged from 2,292 x $(10)^4$ to 26,187 x $(10)^4$.

Testing this difference by the pooled sum of squares method, recommended by Fisher (4) for small samples, gives a value of 3.77 for t. According to Fisher a value of only 2.724 for t is sufficient to denote differences which are highly significant.

These experiments were repeated using 10, 50, 100, 1,500 and 15,000 oöcysts, respectively, the significance of the results of which were statistically tested and summarized in table 3.

TABLE 3. *Significance of susceptibility lost on account of previous infection*

Oöcysts in first infection	No.	Mean yield $(10)^4$	Standard deviation $(10)^4$	Lowest yield $(10)^4$	Highest yield $(10)^4$	Value of t	Value of t above which differences are highly significant
4	19	7,840 ± 1,055	6,805	680	28,368	3.77	2.724
Controls	18	16,525 ± 1,172	7,202	2,292	26,187		
10	15	4,069 ± 680	3,902	221	11,259	4.988	2.763
Controls	15	17,818 ± 1,592	9,135	5,618	33,913		
50	15	1,447 ± 108	625	425	8,300	4.618	2.763
Controls	14	22,379 ± 3,035	9,200	7,978	36,650		
100	14	223 ± 26	144	14	569	4.34	2.807
Controls	11	20,500 ± 3,817	18,730	5,597	58,944		
1500	11	.636 ± 410	2,013	0	7	7.757	2.845
Controls	10	11,259 ± 1,020	4,780	5,069	20,847		
15000	7	——	——	——	——	——	——
Controls	14	10,128 ± 685	3,210	5,221	16,100		

Table 4 shows that there is a conclusive evidence that a single infection produces an appreciable amount of resistance in the body of the host. The question arises as to how much relative susceptibility is lost by the host in infections originating from known numbers of parasites. Furthermore, from the standpoint of the host, what is the optimim dosage for producing a high degree immunity for protection against subsequent reinfection?

In order to get the loss of susceptibility due to the different sized doses in comparable terms, the author has attempted to estimate the percentage of total immunity acquired during the first infection by assuming the controls for each group to be 100 per cent susceptible. The

percentage of immunity acquired was then figured by obtaining the following ratio:

$$\frac{M \times N \times 100}{Mc \times Nc}$$

Where M and N are the mean of yield and number of experimental animals, and Mc and Nc are the mean of yield and number of controls, respectively.

Figuring the percentages in this way, infective doses as small as 4 oöcysts were found to produce as much as 53.02 per cent immunity, while a single dose of 1,500 oöcysts caused a loss of 99.995 per cent of total susceptibility. None of the infections due to 1,500 oöcysts or less seemed to produce any severe clinical symptoms.

Table 4 gives the fraction of total immunity gained by different sized doses. It is of interest to note that while a dose of 1,500 oöcysts produced 99.995 per cent total immunity, doses of 50 and 100 produced almost as much—90 and 98 per cent, respectively. There were in the first group eleven experimental animals, ten of which were completely immunized after the single infective dose of 1,500 oöcysts.

TABLE 4. *Effect of previous infection on oöcyst yield after standard immunizing dose (1,500 oöcysts daily for 5 days)*

Number of oöcysts in infective dose	Mean yield from previous infection	Oöcysts produced per oöcyst fed to experimentals	Oöcysts produced per oöcyst fed to controls	Percentage of total immunity produced by first infection
4	$873 \times (10)^4$	10,452	22,033	53.02
10	$2,084 \times (10)^4$	5,429	23,757	77.15
50	$8,235 \times (10)^4$	1,930	20,000	90.35
100	$15,445 \times (10)^4$	293	27,300	98.03
1,500	$28,830 \times (10)^4$	8	15,012	99.995
15,000	$38,254 \times (10)^4$	0		100.00

4. WEIGHT CHANGES, PATHOLOGICAL EFFECTS AND LETHAL DOSAGE

No apparent clinical symptoms were evident in rats receiving an infective dose of one or four oöcysts. In an infection resulting from ten to fifteen hundred oöcysts, the most noticeable symptoms were general sluggishness and loss of responsiveness to raps on the cage on about the seventh and eighth days after the date of infection. Not until the single infective dose was increased to 15,000 viable oöcysts did the experimental animals gain weight at an apparently less rapid rate than did normal rats during the same period.

In the lighter infections there were days on which there was no appreciable gain, but corresponding weight changes were usually observed in the control animals. The author believes, therefore, that to place too much emphasis on the gain or loss of weight due to the lighter infections

would be misleading since some of the differences might chance to be due merely to physiological coincidents rather than to pathological conditions.

Following an infection with a dose as large as 15,000 oöcysts, however, there was during the infection a percentage gain over the initial weight which appears to be significantly smaller than that of the controls during the same period. The experimental animals in this group weighed an average of 77 grams at the beginning of the infection, and gained during the experiment twenty-three per cent of their initial weight; while the controls weighed only 70 grams to begin with, and before the end of the experiment had increased in weight forty-four per cent. This observation is still more impressive when it is taken into consideration that forty-six per cent of the animals infected with this sized dose died on the eighth day after the date of infection, and that the animals here considered represent the more resistant survivors.

It was found that by feeding 15,000 oöcysts there was a forty-six per cent mortality on the eighth day of the infection, whereas with a larger dose of 1,500,000 oöcysts, ninety-two per cent of the fourteen rats died within thirty-six hours after being infected. All rats in either case developed a severe diarrhea before death, and a post mortem examination showed the small intestine to be markedly hemorrhagic.

SUMMARY AND CONCLUSIONS

1. The mean yields of the oöcysts of *Eimeria miyairii* from single infective doses of different sizes were as follows:

1	oöcyst	62,000
4	oöcysts	8,730,000
10	”	20,840,000
50	”	82,350,000
100	”	154,450,000
1,500	”	288,300,000
15,000	”	382,540,000
1,500,000	”	1,550,000,000

2. The prepatent period for a single infective dose ranging from one to 1,500,000 sporulated oöcysts is approximately seven days. The patent period for the same varies from three to five days, and in the majority of cases is either four or five days. Its length seems to be independent of the size of the infective dose.

3. Different sized infective doses are in no way reliably indicative of a predictable gain or loss in weight during the infection. There is naturally a big variation in the daily gains of weight among rats, but this variation is not in general affected by the coccidial infections produced by from one to 1,500 sporulated oöcysts. Single infective doses of 15,000 oöcysts, however, cause an increase in weight during the infection significantly less than that of the controls during the same period of time. This difference is most manifest during the first nine days of the infection.

4. Single infective doses as small as four viable sporulated oöcysts cause a rat to lose opproximately fifty per cent of its natural susceptibility;

TABLE 5. Effect of different sized infective doses on mean daily gain in weight in grams

Day after infection	Number of oöcysts in infective dose														
	1		4		10		50		100		1,500		15,000		1,500,000
	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
1	5.8		5.5	6.5	4.2	3.0	4.5	5.3	1.4	4.8	3.0	4.7	3.0	2.4	1	3
2	4.5		1.5	1.1	5.5	3.6	3.6	4.3	5.8	3.8	2.8	2.5	3.3	2.9	0	16
3	3.0		2.8	6.1	3.8	4.7	3.6	4.4	4.0	5.3	8.0	6.5	4.6	3.0	2	7
4	3.1		7.8	2.7	3.8	4.5	5.2	3.3	4.7	2.7	2.8	4.0	.8	3.2	7	4
5	3.6		6.4	5.1	6.2	4.8	4.4	3.3	4.8	6.1	—1.2	3.3	5.6	3.0	4	5
6	3.3		3.6	7.6	4.1	3.4	2.9	2.0	3.2	4.0	11.7	6.0	1.8	5.1	3	10
7	2.4		5.0	4.7	2.6	4.3	2.6	4.5	2.6	4.8	—.7	5.5	—2.0	—.2	—2	—2
8	3.8		3.4	1.2	5.7	4.2	1.2	3.5	1.4	3.1	—3.8	—.1	—7.1	2.2	—2	7
9	2.4		3.7	5.9	5.2	4.5	5.2	3.3	3.5	4.0	3.8	4.6	—2.3	1.6	12	2
10	3.0		4.7	2.6	4.2	4.0	5.6	4.5	2.8	5.1	4.2	5.4	3.0	2.0	14	5
11	2.0		2.7	.6	4.0	4.3	4.6	3.5	5.2	.9	6.1	3.6	2.7	2.7	2	2
12	1.8		6.0	6.6	3.0	4.0	3.2	2.4	3.7	5.8	6.5	1.5	2.5	3.8	2	2
Initial weight	100		79	80	79	79	83	75	87	83	85	82	77	70		
Total gain during infection	39		53	51	52	44	47	44	43	50	43	47	18	31		
Percentage gain over initial weight	39		67	63	66	62	56	57	49	60	50	58	23	44		

ten oöcysts, approximately seventy-five per cent; fifty oöcysts, approximately ninety per cent; one hundred oöcysts, approximately ninety-eight per cent; and fifteen hundred, approximately one hundred per cent.

5.   Single infective doses of 15,000 viable oöcysts prove fatal on the eighth day of the infection in approximately fifty per cent of the cases, while after the much larger dose of 1,500,000 sporulated oöcysts there is approximately a ninety-five per cent mortality within thirty-six hours after the time of inoculation.

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ORGANISMS PRODUCING A POTATO ODOR IN MILK[1]

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A potato odor is occasionally encountered in milk and cream. There appear to be at least three possible causes of this condition: (a) holding unprotected milk or cream where there is the odor of potatoes, (b) feeding potatoes to the cows producing the material[2] and (c) the action of microorganisms. Two types of bacteria causing a potato odor in dairy products have been isolated at the Iowa Agricultural Experiment Station. The work herein reported deals with the identification and comparison of these two types.

PSEUDOMONAS GRAVEOLENS

Recently an outbreak of a potato odor in milk from a certain farm over a period of about two weeks was reported by an Iowa milk plant. Inquiry by the plant management revealed that the milk was not exposed to the odor of potatoes and that potatoes were not being fed to the producing animals. A sample of the milk was plated, using beef infusion agar and incubating at 21° C.; after about 15 hours considerable numbers of colonies were evident, and the plates had an odor definitely suggestive of potatoes. On picking cultures into litmus milk, a number of them, all of which seemed to be of the same type, quickly produced a potato odor at 21° C. The odor, which duplicated that in the sample of milk, was very conspicuous and so definite that it was described as resembling potatoes by various persons whose attention was called to it. When there was a number of milk or agar cultures of the organism together, the odor could often be detected on entering the room where they were being held.

A study of the morphologic, cultural and bio-chemical characters of the organism isolated definitely identified it as *Pseudomonas graveolens*, which was described by Levine and Anderson (4) following its isolation from musty eggs. A comparison of the organism with a culture of *Ps. graveolens* used in the preparation of the original description showed that the two were the same, although they differed in the intensity of the potato odor produced. Cultures of the organism isolated were grown in various media and submitted to Levine and Anderson. They considered the potato odor identical with the odor which they had termed musty. The odor-producing characteristic of their culture had apparently been partially lost during numerous transfers on laboratory media.

Ps. graveolens has a number of characteristics that should be considered in connection with its ability to cause odor defects in dairy products. It fails to grow at 37° C., and when plates poured with the defective milk, from which *Ps. graveolens* was isolated, were incubated at this tempera-

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[2]Babcock (1) reported that feeding cows potatoes one hour before milking resulted in only slight abnormal flavors and odors in the milk.

ture the organism did not develop. In a flask of aseptic milk inoculated with the organism and held at about 8° C., the count increased from 12,900 per ml. immediately after inoculation to 5,500,000 per ml., after 96 hours, when the potato odor was very evident. These results indicate that ordinary cooling will not prevent the growth of *Ps. graveolens* in milk. The ability of the organism to grow rapidly at ordinary temperatures may make it a serious contaminant on farms where thorough cleaning and sterilizing of the utensils are not practiced.

On extended holding, cultures of *Ps. graveolens* often developed an odor more like that of turnips than of potatoes.

In order to determine the effects of pasteurization on the potato odor, a small lot of pasteurized milk was inoculated with *Ps. graveolens* and held at 21° C. until a definite potato odor had developed. Half of the lot was then re-pasteurized at 62.8° C. for 30 minutes and compared with the half not pasteurized after inoculation. The comparison showed that the intensity of the odor was not decreased by the pasteurization.

PSEUDOMONAS MUCIDOLENS

In the studies carried out at the Iowa Agricultural Experiment Station some years ago on the changes in flavor and odor that occur in cream held at relatively low temperatures, a sample of cream was encountered that had developed an odor resembling potatoes. An organism that produced a potato odor in milk or cream was easily isolated[3] by plating on beef infusion agar and incubating the plates at 21° C. The odor was very conspicuous and readily recognized. The organism was identified as *Pseudomonas mucidolens*, which was described by Levine and Anderson (4) following its isolation from musty eggs. A comparison of the culture isolated with a culture of *Ps. mucidolens* used in the preparation of the original description showed the two agreed in their general characters, although the culture isolated from cream reduced nitrates more slowly and less extensively, produced hydrogen sulfide more slowly, and hydrolyzed fat more rapidly than the other culture. Moreover, the culture isolated from cream frequently showed several flagella at one pole, while the cultures secured by Levine and Anderson showed a single flagellum.

ADDITIONAL OBSERVATIONS ON PS. GRAVEOLENS AND PS. MUCIDOLENS

From the studies carried out, a number of characters of importance can be added to the published descriptions of *Ps. graveolens* and *Ps. mucidolens*. The former organism did not attack various fats, while the latter hydrolyzed various fats as shown by Nile-blue sulphate and also by the production of rancidity in butter made from cream to which the organism had been added. In some instances *Ps. mucidolens* produced rancidity in milk and cream along with the potato odor; rancidity was very slight in milk, however. *Ps. graveolens* showed a single flagellum, as did the culture of *Ps. mucidolens* secured from Levine and Anderson, while the culture of *Ps. mucidolens* isolated from cream frequently showed several flagella at one pole. Both types grew well in Dunham's peptone solution, but neither showed growth in Uschinsky's solution.

In bouillons (0.5 per cent peptone and 0.3 per cent beef extract) containing one per cent of various fermentable materials, the following reac-

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[3]The isolation was made by M. A. Collins.

tions were secured with *Ps. graveolens* and *Ps. mucidolens* at 21° C., bromcresol purple being used as the indicator. Gas was not produced by either organism. Both types formed acid in two to four days from arabinose, dextrose and galactose, but not from sucrose, maltose, lactose, raffinose, dextrin, starch, inulin, glycerol, dulcitol, mannitol, sorbitol, adonitol, inositol or salicin. From levulose *Ps. graveolens* formed a slight acid reaction after 18 days and *Ps. mucidolens* after 8 days; from rhamnose both types produced a slight acid reaction after 18 days. Using a peptone-dipotassium phosphate medium, with Andrade's indicator, Levine and Anderson (4) secured reactions differing somewhat from the above.

Levine and Anderson (4) found that in litmus milk *Ps. graveolens* showed "reduction and coagulation after two weeks with an acid ring on the surface." With the cultures of *Ps. graveolens* studied, including a culture from Levine and Anderson, the reaction in litmus milk was neutral or slightly alkaline at first, later becoming definitely acid and reduced at the bottom; some of the cultures eventually coagulated the milk.

OTHER ORGANISMS PRODUCING A POTATO ODOR IN MILK OR CREAM

A lot of cream having a potato odor was studied by Brannon (2), who isolated an organism which reproduced the characteristic odor within 24 hours after inoculation into freshly separated cream held at 21.1° C. Inoculated cream incubated at 10.0°, 26.7° or 7.38° C. did not develop the odor. Brannon also mentioned having previously isolated an organism which produced a potato flavor and ropiness in cream; however, this organism soon lost its ability to produce the characteristic flavor.

In further observations on the organism producing a potato odor, Brannon (3) stated that, "If cream in which this organism has grown and produced the potato odor is churned into butter the potato odor persists. This organism has been inoculated into milk which later was converted into other dairy products and if the potato odor appeared in the milk it also appeared in the manufactured product." Brannon did not identify his organism with any of the known species or suggest a name for it.

SUMMARY

Two organisms causing a potato odor in milk or cream have been identified as *Pseudomonas graveolens* and *Pseudomonas mucidolens*. In addition to the production of the potato odor, *Ps. mucidolens* also hydrolyzed fat actively; it produced rancidity in butter, and sometimes in milk and cream.

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CONTENTS

NOTE: Complete copies of these theses can be consulted at the Library, Iowa State College, Ames, Iowa.

THE DETERMINATION OF THE THERMIONIC WORK FUNCTION OF NICKEL BY A NEW METHOD[1]

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The photoelectric work function of nickel has been determined a number of times in the past (1, 2, 3, 4, 5, 6). Due to contaminated surface conditions or poor vacuum technique, the earlier values were too low, ranging from 3.6 volts to 4.5 volts. However, Glasoe (6) in 1931, using Fowler's (7) method of analysis and after a prolonged period of outgassing, obtained the value of 5.01 ± 0.02 volts. The only previous thermionic determination was made by Schlichter (8) in 1915. Because of poor vacuum technique he obtained the erroneous value of 2.77 volts.

It was the purpose of this work to redetermine the thermionic work function of nickel by a new and more suitable method and to compare the result so obtained with that of Glasoe. The development of the new method was made necessary because the conventional method requires a strip of wire of uniform cross-sectional area to be heated by an electric current. During the prolonged outgassing process required, the uneven evaporation of nickel would cause hot spots to develop which would invalidate the results.

The method and the results have been given in a paper by Fox and Bowie (9), the abstract of which is given in the following paragraph.

The metal sample was in the form of an approximate sphere and was heated by electron bombardment from an auxiliary filament which was disconnected when measurements were made. Electron emission from the cooling sample charged a condenser, which, at predetermined times, was discharged through a ballistic galvanometer. Temperatures were determined by a Pt. Pt ± 10 per cent Rh thermocouple spot-welded to the sample. The thermionic constants were obtained from the equation: $\log_{10}(T^2/SQ) = \log_{10}(2.3/aA + Q(1.988 \times 10^{-4}T)$, where Q is the quantity of charge yet to flow upon cooling the sample from a given temperature to absolute zero, —S is the slope of the $\log_{10} Q$ vs. X time curve. This equation is derived from Richardson's, a is the area of the sample, A is the constant in Richardson's Equation, and T is the temperature of the sample in degrees Kelvin. The values of the thermionic constants obtained by applying this method to the case of thoroughly outgassed nickel are found to be Q = 5.03 ± 0.05 volts and A = 1.38 × 10³ amp/cm² deg.².

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THE USE OF POLYMERS FROM FURFURAL IN THE FABRICATION OF MOLDED PRODUCTS[1]

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The addition of sulphur or hydrochloric acid to a 20 per cent solution of furfurin in furfural produces a black, resinous body. This material is quite acidic, due to the acid used to produce the polymerization, and has a tendency toward shrinking and cracking. This plastic has been made the subject of a series of experiments in an endeavor to find some method by which the defects of the material could be corrected, and to adapt the plastic to the fabrication of molded products.

The treatment of an aqueous solution of furfural with hydrogen sulphide results in the formation of a colloidal solution of furfural polysulphide. Experiments were conducted to determine if the treatment of pure furfural with hydrogen sulphide could be used in producing a molding material. A black, hard, shiny resin was produced by bubbling hydrogen sulphide through furfural at 110° C. for one hour, and polymerizing this solution by adding 15 parts of concentrated hydrochloric acid to 100 parts of H_2S-furfural by volume. This material had a tensile strength of 800 pounds per square inch, but cracked shortly after removal from the mold. A mixture of furfurin solution and furfural treated with hydrogen sulphide was used. A mixture of 50 parts (by volume) of furfural treated with hydrogen sulphide for one hour, 30 parts of furfurin solution, and 20 parts concentrated hydrochloric acid resulted in a material having a tensile strength of 850 pounds per square inch. This compound also cracked after removal from the mold. The cracking can be prevented by coating the articles with paraffin, so as to prevent contact with the air.

Numerous compounds were tested to determine their ability to cause catalytic polymerization of the furfurin solution, and to discover if their addition would produce a material less susceptible to cracking. Of these compounds, sodium tungstate and gallic acid produced a slightly more rapid solidification. The addition of small amounts (2 per cent by weight) of ferrous ammonium sulphate decreased the cracking tendency slightly, as did manganous and ammonium chlorides. Silicon tetrachloride was tested as a polymerizing agent. A hard, very brittle resin resulted when 8 parts of silicon tetrachloride was added to 100 parts of furfurin, by volume. The polymerizing effect of the silent electric discharge did not produce a solid material.

The introduction of anhydrous ammonia into furfural is accompanied by an increase in the specific gravity of the solution. This increase of specific gravity is of interest, since it permits a determination of the concentration of the furfurin solution by obtaining the specific gravity and reading the corresponding concentration from a suitable table.

The properties of many resins are improved by the addition of a plasticizer. Dibutyl phthalate was tested as a plasticizer. Due to the compound's immiscibility with the aqueous acid used, the phthalate separated from the mixture. Non-aqueous solutions of hydrochloric acid, such as an alcoholic solution, were tried. The resins produced using furfurin solution, dibutyl phthalate, and a solution of hydrochloric acid in butyl alcohol were hard, glossy, and had a lower density than the furfurin-solution-aqueous hydrochloric acid resins. A slight tendency to crack was observed, although in numerous instances samples were obtained which were free from cracking.

Acetone was substituted for butyl alcohol as a solvent for hydrochloric acid gas, with excellent results. The freedom from cracking of these materials was a quality which had not been observed in any other resin. Sulphuric acid was mixed with the acetone, and the mixture added in various proportions to the furfurin solution. An acetone-sulphuric acid mixture of 80 parts sulphuric acid to 100 parts acetone by volume produced excellent results when added to the furfurin solution in the ratio of 20 parts of acetone-acid mixture to 100 parts of furfurin solution.

A step farther in the use of acetone was to reflux the acetone and furfurin solution, and add acid to the refluxed mixture. The final formula developed for producing the resin is: (1) Reflux a mixture of 10 parts of a 20 per cent furfurin solution and 1 part acetone (by volume) for 20 minutes. (2) Cool to 30° C. and add slowly with stirring 1 part of concentrated sulphuric acid to 10 parts (by volume) of the refluxed mixture. Cool rapidly to 35 ° C., add fillers if desired, and place in molds. (3) Remove from molds as soon as solid enough to permit handling. Cracking of the material results if the article is permitted to remain in the mold for an extended period of time. The resin produced has a tensile strength in excess of 1,000 pounds per square inch. It is hard, glossy, does not crack, and is easily molded.

The refluxed acetone-furfurin solution plastic was used in the fabrication of small tanks. In this application of the resin 17 grams of shredded asbestos per 100 cubic centimeters of liquid material was used as a filler. A small size, the inside dimensions of which were $7\frac{1}{2}$ x $4\frac{1}{2}$ x $4\frac{1}{2}$ inches, with half-inch walls, was first molded; later a larger tank, 18 x 6 x 6 inches, with a wall thickness of three-quarters of an inch, was produced. The outstanding feature of these tanks was their resistance to corrosion. Aqueous solutions of caustic, and sulphuric and hydrochloric acids did not corrode the material. Aqueous hydrofluoric acid was also kept in the tanks without damage by the acid. The resinous material did absorb some of the liquid; when the tanks were emptied and dried, the evaporation of the absorbed solution caused the walls of the tanks to warp and crack. This defect can be avoided by keeping the tanks filled at all times.

The addition of sulphur dioxide to furfural results in a solution which is polymerized to a soft, porous material by the addition of hydrochloric acid. The softness of the material enables it to be used as a crayon. The optimum amount of sulphur dioxide was found to be 8 grams per 100 grams of solution. The correct amount of hydrochloric acid is dependent upon the hardness desired; an increase in the amount of acid used decreases the hardness. The addition of furfural treated with oxy-

gen was found to be beneficial. The treatment with oxygen consisted of bubbling the gas through furfural heated to a temperature of 110° C. An excellent group of crayons may be produced by mixing 6 parts of an 8 per cent sulphur dioxide in furfural solution with 4 parts by volume of furfural treated with oxygen for 2 hours. This mixture is polymerized with amounts of concentrated hydrochloric acid varying from 10 to 100 per cent of the amount of furfural mixture used. By varying the acid used in 10 per cent intervals, a series of ten crayons having an excellent gradation in hardness is obtained. The crayons compare favorably with the charcoal pencils used for sketching purposes.

A plant for the production of the refluxed acetone-furfurin solution plastic has been designed. The plant is divided into two units, the first of which produces the furfurin solution, and refluxes it with the correct amount of acetone. The second unit is designed to mix continuously the sulphuric acid and refluxed acetone-furfurin solution in the desired ratio, and to cool the mixture rapidly to its molding temperature. Mixers are provided for the incorporation of fillers.

QUANTITATIVE STUDIES ON THE FORMATION OF XYLOSE FROM PENTOSAN-CONTAINING MATERIALS[1]

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The utilization of agricultural products for the production of industrial chemicals is a subject of paramount interest in the field of chemical research at the present time. The pentosan-containing materials may be considered as by-products from the various agricultural industries. Some of these by-products which are produced in abundance include bagasse, cornstalks and cobs, cereal straws and hulls, peanut shells, cotton burs, and cottonseed-hulls. These materials, upon acid hydrolysis, yield up to 40 per cent reducing sugars, chiefly xylose. The purpose of this investigation was to make a study of the effect of acid concentration (HCl) and temperature (steam pressure) on the rate of hydrolysis and yield of xylose from oat-hulls, and to correlate the copper number with the production of solvents by the butyl-acetonic fermentation.

EXPERIMENTAL

ANALYTICAL METHODS

The reducing sugars formed by dilute acid hydrolysis of the pentosan-containing materials (oat-hulls) were determined by the method of Shaffer and Hartman.

APPARATUS

The apparatus consisted of a specially built autoclave (digester), constructed in such a manner that samples could be removed from the reaction medium for analysis without disturbing the temperature and pressure equilibrium. The essential parts of the digester were: (1) the reaction chamber which consisted of a five liter balloon flask; (2) motor driven mechanical stirrer; (3) small condenser connected to a sampling device; (4) a shell or autoclave that would operate under steam pressure up to 100 pounds per square inch.

SUMMARY OF RESULTS

The optimum conditions for hydrolysis of oat-hulls by dilute hydrochloric acid at various pressures are presented in the following table.

Studies were made on the liquid-solid ratio (cubic centimeters HCl solution per gram of oat-hulls) at 40 pounds steam pressure per square inch. It was shown that with increase in concentration of hulls there is a drop in yield of reducing sugars, and the time lag increases. That is, with increase in concentration of hulls the acid concentration must also be increased. For example, with a 5:1 ratio the optimum acid concen-

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TABLE 1. *Optimum conditions for hydrolysis of oat–hulls with hydrochloric acid at various pressures*

Liquid–hull Ratio	Pressure Lbs./Sq. In.	HCl normality	Time of heating in minutes	Reducing sugars percentage
10 to 1	Atmospheric	2.000	180	39.50
10 to 1	20	0.100	120	40.00
10 to 1	40	0.050	90	40.00
10 to 1	60	0.042	75	39.50
10 to 1	80	0.042	60	39.90
10 to 1	100	0.042	30	40.50
5 to 1	40	0.070	150	36.00

tration at 40 pounds is 0.02 N greater than that for a 110:1 ratio at the same temperature. The maximum yield for a 10:1 ratio was 40 per cent xylose, and that for a 5:1 ratio was found to be 36 per cent. The corresponding xylose concentrations are 4 per cent and 7.2 per cent, respectively.

A study was made of the carbon balance during hydrolysis of the oat-hulls. The data show that, during hydrolysis, about 45 per cent of the hull is dissolved with a yield of 39.1 per cent reducing sugars (xylose); the remaining unhydrolyzed residue amounted to 52.6 per cent. About 2.2 per cent of volatile products was lost during the hydrolysis. The hydrolysis was carried out under the following conditions: 20 pounds steam pressure per square inch, 10.1 ratio, 0.1383 N HCl, time of cook 90 minutes. The oat-hull and residue, after hydrolysis, were both analyzed for lignin, to determine its distribution. About 21.7 per cent of the lignin was dissolved or decomposed during hydrolysis. The distribution of ash was accounted for quantitatively. The residue after hydrolysis of the oat-hull was shown to consist mainly of lignin, crude fiber, and ash; these values total to 99.56 per cent. The oat-hulls were also analyzed for moisture loss at 105° C., ether solubles, and crude fiber. These data from the carbon balance studies indicate that xylose results from the hydrolysis of a C_5 compound rather than from a C_6 compound, and that volatile acids, particularly acetic, are formed in appreciable amounts.

Xylan was prepared from clean oat-hull meal, by extraction with 7 per cent sodium hydroxide solution and subsequent precipitation of the xylan by the addition of two volumes of 95 per cent alcohol. After filtration, the xylan was washed with alcohol and ether, dried in the air and stored in a tightly stoppered bottle. The xylan was analyzed for ash, moisture, lignin, and xylan by hydrolysis to xylose; the lignin was determined by difference. The xylan was hydrolyzed in the digester under similar conditions to those employed for the oat-hull hydrolysis. The results show that xylan is more readily hydrolyzed in the free state by dilute hydrochloric acid solution than when present in the hulls.

A correlation was made between the copper number and the production of solvents by the butyl-acetonic fermentation. Crude xylose solutions were prepared by hydrolyzing oat-hulls with 0.07 N HCl solution under 20 pounds pressure per square inch in the digester. A 5:1

ratio was used and a series of cooks were made (60, 90, and 150 minutes). The pH of these crude xylose solutions was regulated to 5.0 by the addition of saturated NaOH solution, and the quantity of reducing sugars (copper number) present, was determined. These solutions were then fermented and analyzed for total solvents according to the standard technique employed in this laboratory. The data indicate that the maximum yield of solvents is produced from solutions which have had less drastic treatment than is required for the maximum yield of reducing sugars. That is, the best results in fermentation will be obtained when the acid concentration, temperature, or time of hydrolysis are slightly less than those given for maximum yield of reducing sugars.

CONDENSATION REACTIONS OF FURFURAL AND ITS DERIVATIVES[1]

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PART I

POLYMERIZATION AND STABILITIES OF FURAN COMPOUNDS

Preliminary to investigations on actual condensation reactions, a series of studies was made on the stabilities of furfural and its derivatives.

By treating 1 cc. portions of furfural with one drop or a small fragment of 66 different inorganic reagents, it was found that the greatest decomposition and resinification was produced by the halides of elements which occur in the center of the Periodic Table. This decomposing activity was particularly noticeable with the halides of the Groups 3, 4 and 5.

In general, organic compounds were without marked action unless an active halogen or strongly acidic group were present. Amino compounds caused a slow decomposition. Furfuryl alcohol behaved in a manner similar to that of furfural.

Hydroquinone, pyrogallol and pyrocatechol previously recommended[2] as stabilizers for furfural and certain furan compounds were found to be not only without stabilizing effect, but they actually accelerated decomposition of furfural when the furfural was sealed for three years with the so-called stabilizers. When the tubes of furfural with a little stabilizer were left open to the acidic atmosphere of the laboratory, pyrogallol, hydroquinone and pyrocatechol[3] were found of value as stabilizers.

The best method for keeping furfural is to seal it in an inert atmosphere. It was found that furfural sealed with nitrogen kept for three years. It became brown in color.

Furfuryl alcohol sealed with air became only a golden-yellow in color after three years standing.

In a series of experiments, 34 different compounds were treated separately with each of the following reagents: phosphorus tribromide, silicon tetrachloride, phosphorus trichloride, sulfur monochloride and arsenic tribromide. Varying degrees of stabilities were noted. The influence of a miscellany of substituents in the furan ring was noted.

The interesting fact was noted that the more sensitive groups are, in general, those that contain a methylene group attached to the furan

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[1] Original thesis submitted December, 1933. This work was directed by Dr. Henry Gilman.

[2] Moureu, Dufraisse and Lotte, *Compt. rend.,* **180**:993 (1925); Moureu and Badoche, *ibid.,* **187**:157 (1928; Moureu and Dufraisse, *Chem. Ind.,* **47**:819 (1928); Moureu, Dufraisse and Johnson, *Bull. soc. chim.,* **43**:586 (1928).

[3] Other substances were also studied in regard to their stabilizing effect.

nucleus. The greatest stabilizing groups are nitro and carboxyl and groups derived from carboxyl.

COLOR REACTIONS

An attempt was made to obtain a satisfactory color reaction for the furan nucleus. A color reaction would give a means of readily determining the presence of the furan ring after a series of vigorous transformations. The tests with aniline acetate[4], vanillin[5], pine splints[6], isatin[7] and ferric chloride[8] have been found essentially unreliable and insufficient.

It was hoped that in the various treatments of furan compounds with various substances a satisfactory color reaction would result. No definite or uniform color was observed. Another attempt along similar lines has recently been reported[9].

PART II

The actual condensation reactions studied for furan compounds were the Friedel-Crafts and Gattermann-Koch reactions.

The first definite work done in the introduction of acyl groups by the Friedel-Crafts and Gattermann-Koch reactions was reported by Reichstein[10] as recently as 1930. No attempt was made to alkylate furan compounds prior to a quite recent paper on the alkylation and acylation of furan compounds[11]. This earlier paper[11] reported the successful alkylation of alkyl furoates, furyl ketones and furfural. This latter substance gave an anomolous product that is being further investigated. It was found[11] that alkyl furoates and furan could be acylated in good yields. Ethyl 2-methyl-5-furoate has been acylated[12].

DISCUSSION

Furan compounds, in general, show a greater ease of alkylation and acylation than than benzene or benzene compounds[11].

The 2-furyl alkl ketones prepared as recently directed[11] showed no hypnotic action. Those tested were furyl methyl, ethyl isopropyl, *n*-propyl, *n*-butyl, isobutyl and *n*-amyl ketones.

The 5-alkyl-2-furoic acids[11] showed marked germicidal action[13]. The acids studied were 5-methyl-, 5-isopropyl-, 5-*tert*.-butyl- and 5-amyl-furoic acids.

Acetylation of 3,4-dicarbomethoxyfuran yielded 2-acetyl-3,4-dicarbomethoxyfuran.

[4]Middendorp, *Rec. trav. chim.*, **38**:47 (1919).

[5]Asahina *et al*, *Acta Phytochim.*, 2:22 (1924); *Chem. Zent.*, **95** (13):1694 (1924).

[6]Reichstein, *Helv. Chim. Act*, **15**:1110 (1932).

[7]V. Meyer, *Ber.*, **16**:1477 (1883); Yoder and Tollens, *Ber.*, **34**:3461 (1901).

[8]Sohst and Tollens, *Ann.*, **245**:20 (1888); Reichstein and Zschokke, *Helv. Chim. Act.*, **15**:265 (1932).

[9]Levine and Richman, *J. Biol. Chem.*, **101**:373 (1933).

[10]Reichstein, *Helv. Chmi. Act*, **13**:356 (1930).

[11]Gilman and Calloway, *J. Am. Chem. Soc.*, **55**:4197 (1933).

[12]Gilman and Calloway, *J. Am. Chem. Soc.*, **56**:0000 (1934) (January).

[13]Unpublished work.

All attempts to carry out a Friedel-Crafts substitution on a furan containing the nitro group were futile. In one case it was found possible to substitute a chlorine atom for the nitro group. That is, from nitro-furan, propionyl chloride and titanium tetrachloride in carbon disulfide there was isolated 2-chlorofuryl 5-ethyl ketone.

The general order of strength of various condensing agents in introducing alkyl groups into the furan nucleus by the Friedel-Crafts reaction is as follows:

$$AlCl_3 > FeCl_3 > SnCl_4$$

The relative arrangement with respect to acylation is the following:

$$SnCl_4 > FeCl_3 > AlCl_3 > TiCl_4$$

EXPERIMENTAL

The technique used throughout was that recently reported[11]. All alkylations, acylations by acid halides and acylations by acid anhydrides were carried out by the same general directions.

The 2-acetyl-3,4-dicarbomethoxyfuran from 3,4-dicarbomethoxy-furan with acetic anhydride and stannic chloride in benzene melted at 108°.

Methyl furoate was alkylated by butylene to yield methyl 5-*tert.*-butyl-2-furoate.

The Gattermann-Koch reaction[14] would not introduce a formyl group into 2-methyl-3-furoic acid or ethyl 2-methyl-3-furoate. This was predicted by the work of Reichstein[14].

SUMMARY

1. The stability of a furan compound depends on the groups present.

2. The value of stabilizing agents depends on the conditions under which furanic substances are stored.

3. The Friedel-Crafts reaction will apply to certain furan compounds.

4. The Gattermann-Koch reaction did not introduce a formyl group into 2-methyl-3-furoic acid or ethyl 2-methyl-3-furoate.

[14]Reichstein, *Helv. Chim. Act*, **13**:345 (1930).

BACTERIOLOGICAL STUDIES ON BUTTER SHOWING SURFACE TAINT[1]

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Surface taint is a butter defect that has been encountered so frequently it has been characterized as a definite abnormality and differentiated from other types of deterioration. It was first brought to the attention of the trade in Western Canada in 1919. What is essentially the same defect has been discussed in other countries under various names. Surface taint is a defect that develops after the butter is made and makes its first appearance at the surface of the butter and then gradually penetrates to the center. The odor and flavor of the defective butter suggest putrefaction. The taint ordinarily appears in the butter about ten days after manufacture when stored at a temperature of approximately 5° C.; at higher temperatures it may be evident in a few days.

The investigation showed that the numbers of bacteria, yeasts and molds in commercial surface taint butter were generally high and they were usually greater at the surface than in the interior of the butter. Many of the microorganisms were essentially the same as those from normal butter. In general, the types of bacteria found in surface taint butter were not unusual. Although the nature of the defect suggests proteolysis, a comparatively small percentage of these types of organisms were isolated by the methods used.

Acidity and lactose determinations of surface taint butter did not show any differences from those on normal butter. Soluble and amino nitrogen determinations of the surface and interior of the defective butter showed evidence of proteolysis. The results from steam distillations indicated that distillates secured from surface taint butter have a greater alkalinity than those from normal butter.

Surface taint butter inoculated directly into normal butter failed to reproduce the defect, but when inoculated into sweet cream, which had been previously pasteurized, and the cream churned, the resulting butter developed the taint in many instances when stored for three days at 15.5° C., or seven days at 5.5° C. By following this procedure the taint could often be reproduced down through a series of churnings.

The cultures to be studied in detail were selected on the basis of the production in milk of an odor that suggested proteolysis. The procedure of inoculating the pasteurized cream and churning it into butter proved to be the only effective means of reproducing the taint. An organism, tentatively designated *Achromobacter putrefaciens*, was isolated from several samples of surface taint butter. *A. putrefaciens* produced typical surface taint in experimental churnings of butter made from cream inoculated with it. This organism was apparently present in only small numbers in the butter from which it was isolated. Also, the organism

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[1]Original thesis submitted June, 1931.

produced surface taint in butter when the numbers per milliliter were comparatively small. Organisms other than *A. putrefaciens,* which would produce surface taint, when inoculated into pasturized cream and the cream churned, were secured from a number of samples of surface taint butter. These organisms appeared to represent three other typs and were tentatively designated types B, C and D. The study made indicates that type D belongs to the genus Achromobacter, while types B and C may possibly belong to the genus Pseudomonas.

Studies made with *A. putrefaciens* and one other type showed that their development, and consequently the development of surface taint, could be controlled by the amount of salt incorporated in the butter. Unsalted and low salted butter always developed the taint, while medium and high salted butter remained normal. Also, the use of butter culture was influential in restraining the development of surface taint. The butter culture was never permitted to ripen the cream, but 10 per cent was added just before churning. The heat resistance of *A. putrefaciens* and the other cultures studied showed that the ordinary temperatures of pasteurization were effective in killing the organisms.

STUDIES ON VITAMINS B AND G IN GROWTH AND LACTATION IN THE RAT

(a) THE EFFECTS OF EXTRACTS OF VITAMIN B AND G
(b) THE DISTRIBUTION OF VITAMIN G[1]

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Experiments performed by Guest, Nelson, Parks, and Fulmer (1), Taylor (2) and investigators in other institutions have demonstrated that certain purified diets, which support normal growth in young rats, fail to supply enough of the water soluble vitamin B complex for lactation. Evans and Burr (3) and Sure (4) observed that three to five times as much yeast was required to supply the vitamin B complex for lactation as was necessary to produce normal growth in young rats. The experiments reported in this paper were designed to investigate the requirements of vitamins B and G during lactation and the distribution of vitamin G in some natural foods.

The basal ration used throughout these studies contained: casein 18, salts (185) 3.7, butterfat 4.0, cod-liver oil 1.0, and dextrin to 100 parts. If the material being studied was fed as a certain percentage of the ration, the dextrin content was reduced.

Pregnant females transferred from the stock ration to the experimental ration on the day of parturition were used in all lactation studies.

Several extracts and concentrates of vitamin B were made from dried yeast, wheat germ, and rice polishings by extraction with various mixtures of water and alcohol. Of the several vitamin B concentrates prepared an activated fuller's earth product, prepared as described below, was found to be the most satisfactory source of vitamin B to be used in lactation studies and in testing the vitamin G content of natural foods and vitamin G concentrates.

Ether extracted rice polishings were extracted with 95 per cent ethyl alcohol acidified with acetic acid. The extract was concentrated under reduced pressure. Lead acetate was added to give the maximum precipitate. This precipitate was filtered off and H_2SO_4 added to the filtrate to precipitate the lead. The pH of the lead free filtrate was adjusted to 4.5 and the vitamin B adsorbed on fuller's earth; so that one gram of fuller's earth was equivalent to 37.5 g. of rice polishings. Young rats grew at a normal rate with 0.053 g. of this activated fuller's earth as the source of vitamin B. Fifty-three thousandths of a gram of activated fuller's earth plus the basal ration produced an average gain of 2.5 g. per rat per week. In view of the findings of Evans and Lepkovsky (5) it is possible that the basal ration supplied some vitamin G; and the vitamin G content of the activated fuller's earth is less than is indicated by the gain of 2.5 g. per rat per week.

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[1]Original thesis submitted July, 1934.

Wilkinson and Nelson (6) observed that hog liver supplemented soybeans for lactation. Guha (7) found an aqueous ox-liver extract to be a potent source of vitamin G relatively deficient in vitamin B. Minced hog liver was extracted by the method used by Guha (7). The aqueous liver extract was concentrated in vacuo to 150 ml. per kilogram of liver. The effect of adding alcohol to this liver concentrate was studied. If ethyl alcohol was added to give a concentration of 50 per cent alcohol, little or no vitamin G was precipitated. As the concentration of alcohol was increased above 50 per cent the amount of vitamin G contained in the filtrate decreased, and the amount of vitamin G in the precipitate increased. The filtrates from precipitation with 80 or 90 per cent alcohol contained little or no vitamin G. The precipitates were potent sources of vitamin G, sufficiently free of vitamin B to produce polyneurities in rats.

Dried hog liver, aqueous extract of hog liver, or the precipitate formed by adding alcohol to a hog liver extract to give a concentration of 80 per cent alcohol were excellent sources of vitamin G. Whey powder and dried yeast were good sources of vitamin G. Wheat germ and rice polishings at a level of five per cent of the diet do not furnish sufficient vitamin G for normal growth. Wheat germ is a more potent source of vitamin G than rice polishings.

Wheat, oatmeal, yellow corn, white corn, and barley at a 60 per cent level, and rice polishings, rice bran, and wheat germ at a 10 per cent level are deficient in vitamin G for lactation. Ten per cent autoclaved yeast supplemented the various seeds and products from seeds to produce lactation at a superior rate. Dried hog liver at a level of 3.3 per cent supplements 50 per cent yellow corn as a source of vitamins B and G for lactation.

Wheat and yellow corn are supplemented by the precipitate formed by adding alcohol to a liver extract to give a concentration of 80 per cent alcohol. Activated fuller's earth (source of vitamin B) does not supplement a wheat or corn ration for lactation. Females on rations in which 50 per cent yellow corn or 10 per cent rice polishings furnish the sole sources of vitamin B complex are unable to rear normal young. The feeding of a hog liver concentrate (source of vitamin G) to the young from the fourteenth to twenty-eighth day increased the weight of the young and decreased the mortality. These facts indicate that the females secreted milk and cared for their young, but the young failed to thrive, due to the low vitamin G content of the milk.

Dried hog liver, the precipitate formed by adding alcohol to an aqueous liver extract to give a concentration of 80 per cent alcohol, or autoclaved yeast are unable to support lactation as the sole sources of vitamins B and G. Activated fuller's earth (source of vitamin B) was also unable to support lactation as the sole source of the vitamin B complex. In order to obtain lactation at approximately the normal rate, it was necessary to feed sources of vitamins B and G simultaneously and at about six times the rate required to produce normal growth in young rats. These data show that increased amounts of both vitamins B and G are required by the lactating female, in order to rear normal young.

In no case was lactation as successful when extracts and concentrates served as the sources of the vitamin B complex as when grains

plus autoclaved yeast, dried hog liver, or the precipitate formed by adding alcohol to a hog liver extract served as the sources of the vitamin B complex. Since these are the same sources of vitamin G as were used to supplement the fuller's earth, it is probable that the grains studied furnished some substance necessary for lactation in the rat that is not extracted with alcohol and adsorbed on fuller's earth.

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SOME FACTORS AFFECTING THE GROWTH OF PENICILLIUM ROQUEFORTI IN CHEESE[1]

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The manufacture of varieties of blue veined cheese, such as Roquefort and Wensleydale, originated as an art which has not been entirely successfully transplanted to other parts of the world. Therefore, a fuller knowledge of the factors which affect the growth of *Penicillium roqueforti* may put the manufacture of such cheese on a more scientific basis.

PART I

THE CITRATES OF MILK AND THEIR POSSIBLE FERMENTATION PRODUCTS AS THEY AFFECT THE GROWTH OF P. ROQUEFORTI

Studies were carried out on the effect of citric and acetic acids on the growth of strains of *P. roqueforti* isolated from Roquefort and Wensleydale cheese. In making these studies the cultures of the molds were inoculated into sweet skim milk and on plates of standard agar containing 0.272 and 0.1423 per cent of citric acid or 0.1313 and 0.048 per cent of acetic acid. The amount of growth in the milk media was determined by the amount of undigested casein present after 10 days incubation; while the diameter of a single colony on the plate, after comparative periods, was used as the index of growth on the agar. The incubation temperature ranged from 20° C. to 22.5° C.

Citric acid and acetic acid, in amounts comparable with those found in milk and starter, had an effect on the growth of different strains of *P. roqueforti*. In milk low concentrations of acetic acid tended to reduce the digestion of casein by strains of *P. roqueforti,* while citric acid tended to increase this digestion. On the other hand, in standard agar acetic acid increased the growth while citric acid tended to inhibit it. This work would indicate that the type of starter used in the manufacture of blue veined cheese might have a significant bearing on the subsequent growth of the mold in the cheese.

An investigation into the proportion of the citrates of milk incorporated in the curd during cheese making showed by analysis (using the method of Beau modified by Denige) of both milk and whey that there were not citrates in the cheese other than those associated with the whey incorporated. Citrates added to the milk for cheese making were found in the whey and not held by the curd. Therefore, the very small propor-

tion of citric acid or its decomposition products in the cheese is unlikely to be of significance in the ripening of cheese.

PART II

THE EFFECT OF AMMONIUM SALTS ON THE GROWTH OF P. ROQUEFORTI IN CHEESE

The work of Weisbrodt showed that NH_4Cl greatly increased the growth of *P. roqueforti* on standard synthetic media. Therefore, determinations of the ammonia in cheese were conducted according to a modification of Lisk's method. This method proved to be satisfactory since there was an almost complete recovery of the ammonia salts added to the cheese. Wensleydale cheeses of various ages were analyzed for ammonia. Very little ammonia was found in the fresh cheese, but a considerable quantity developed in the mature cheese.

Additions of NH_4Cl to the curd at salting, in the approximate proportions as found satisfactory by Weisbrodt in synthetic media, had a detrimental effect on the growth of *P. roqueforti*.

PART III

THE OXYGEN REQUIREMENT OF P. ROQUEFORTI IN CHEESE

The need of oxygen for the growth of *P. roqueforti* having been established by various investigators, experiments were conducted to determine what physical means of increasing the air supply in the Wensleydale could be used to hasten and increase the growth of *P. roqueforti*. A method of putting tubes into the cheese and sucking air into the cheese from the outside was attempted. The method did not prove to be satisfactory, owing to air leaking down on the outside of the tube when suction was applied. In general, good mold growth did not develop in the cheese to which suction had been applied, though in many cases mold had grown in the cheese around the tubes.

A method of forcing oxygen from an oxygen cylinder into the cheese, using the above method of putting tubes itno the center of the Wensleydale cheese, was tried. Though some of the cheese developed mold, the method in general does not point to a satisfactory way of increasing the growth of *P. roqueforti* in cheese.

On the basis of Henry's law, on the relationship of pressure to the solubility of gases in water, a method of frequently changing the pressure of the air on a series of cheese in an enclosed cylinder was experimented with. Seven Wensleydale cheese which were submitted to an average reduced pressure of 616 mm. for 7 hours each day, twenty-three times in all, showed a slight mold growth in three cheese, while the control cheese showed no growth. A record of the CO_2 collected from the above cheese is given and shows that an average of about one gram of CO_2 was removed during each operation.

A second experiment with the same apparatus was conducted in which seven lots of cheese were divided into four groups:

A. Control.

B. Subjected to reduced pressure twice a week for six weeks.

C. Bandages removed and subjected to reduced pressure twice a week for six weeks.

D. Bandages removed and the cheese skewered from one end (28 holes, 1/16 of an inch) and then subjected to reduced pressure twice a week for six weeks.

Two significant results were obtained. The alternation of reduced and atmospheric pressure hastened the mold growth but did not permanently improve it.

Skewering the cheese produced a permanent increase in mold growth for the cheese in Group D, as shown by final scores.

A preliminary experiment to determine the possibility of reducing the CO_2 produced in the cheese by fermentation was conducted. The percentage of the sugar incorporated in the curd was lowered by adding acidified water to the milk. Interesting results affecting the process of manufacture of the cheese are given.

Although the cheese had not had time to develop mold when the results were recorded, it was noted that the control group was more acid in taste and firmer in texture than those made with diluted milk. The cheese made with diluted milk appeared to be of a type that would ripen to a more satisfactory Wensleydale cheese.

CAUSES OF SLOW ACID PRODUCTION IN BUTTER CULTURES[1]

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The object of the investigation was to determine the reason why some butter cultures, which appear to be normal on the basis of chemical, bacteriological and organoleptic tests, fail to produce acid at a normal rate when inoculated into freshly pasteurized milk. This abnormally slow acid development in butter cultures is apparently encountered wherever butter cultures are used. The results obtained in the investigation are presented in two parts.

Part I deals with the investigation of factors closely associated with the milk as a cause of slow acid development. The following factors were studied: (a) source of the milk, (b) organisms present in the milk, and (c) contamination from plant equipment.

The source of the milk had little effect on the time required for coagulation by butter cultures or *Streptococcus lactis* cultures, whether the milk was raw or pasteurized. This was the case with both herd milk and the milk from individual cows. The variations were so small that the source of the milk was considered unimportant as a cause of slow acid production in butter cultures. The greatest variations were those caused by pasteurizing the milk and by using different butter cultures and *S. lactis* cultures. Pasteurizing the milk at 82° C. for 30 minutes decreased the time required for coagulation about 30 minutes as compared with raw milk. The difference in coagulation time among various butter cultures and also among various *S. lactis* cultures was as much as six hours.

The organisms present in the samples of raw milk tested did not restrain the development of acid by a butter culture to any marked extent. Furthermore, six unidentified cultures isolated from the raw milk did not have any significant restraining action on the production of acid by a butter culture.

Contamination from plant equipment, with the samples of milk studied, was not important as a cause of variations in acid production by butter cultures. The variations in the samples were too small and too inconsistent to be of any significance. However, it was in connection with the study of contamination from plant equipment that large differences were first noted in the rates of acid production by apparently normal butter cultures. These differences, caused by exceptionally slow rates of acid production by large lot cultures as compared with mother cultures, led to a study of the butter cultures used as inoculating material.

Part II, dealing with the investigations of the butter cultures used as inoculating material, involved (a) examination of butter cultures, (b) examination of bacteria free filtrates from butter cultures, and (c) attempts to produce slow butter cultures experimentally.

In the examination of butter cultures, it was found that the growth of some freshly inoculated butter cultures could be definitely restrained by the addition of small amounts of certain large lot cultures or mother cultures. This restraining effect was a more common characteristic of abnormally slow butter cultures (failing to show coagulation after 16 hours) than of normal cultures. Furthermore, a restraining effect was shown by a larger proportion of the large lot cultures studied than of the mother cultures. No contamination was found in the 23 slow acid producing cultures or in the 17 normal cultures studied (some of each group being inhibitory) by plating on tomato juice agar or by microscopic examination. The morphology of the cells appeared to be typical of butter culture organisms.

Bacteria free filtrates[2] were obtained from butter cultures by filtering them through coarse filter paper, then passing the filtrates through grade N Berkfeld filters. Tests for sterility of a considerable number of the filtrates were made by inoculating one ml. into each of two tubes of sterile litmus milk and incubating one of the tubes at room temperature and one at 37° C., and also by making smears of the filtrates on two plates of beef infusion agar and two plates of tomato juice agar and incubating one of each of these at room temperature and one at 37° C. The litmus milk tubes did not show changes except in one case in which a slow acid development appeared. The contents of many of the tubes showing no changes were examined microscopically and no indications of growth were ever found. The agar plates did not show growth of any nature after four days incubation, except that on an occasional plate a mold colony developed.

When bacteria free filtrates obtained from certain slow and normal butter cultures were added to freshly inoculated portions of a butter culture or *S. lactis* culture there was a definite restraining action on the development of acid and on the increase in the numbers of bacteria. Since 11 (58 per cent) of the 19 filtrates from the mother cultures and 22 (96 per cent) of the 23 filtrates from large lot cultures caused rather marked decreases in the percentages of acid produced by the test cultures, it appeared that large lot cultures were more likely to yield restraining filtrates than mother cultures.

When plates were poured with butter cultures or *S. lactis* cultures containing bacteria free filtrates and colonies picked into tubes of litmus milk, the coagulation rates of the *S. lactis* cultures appeared to be normal, and in the case of the butter cultures, there seemed to be a normal distribution of the organisms among the butter culture types. Carrying mixtures of a butter culture and each of a number of restraining filtrates through a series of seven transfers commonly restored the culture to a normal rate of growth, and attempts to increase the activity of filtrates by adding them to a butter culture and then recovering them when coagulation had occurred were unsuccessful. Heating to comparatively low temperatures for short periods seemed to destroy the restraining action of the filtrates.

Since it was found that large lot cultures, which are exposed to the air more than mother cultures in the process of making, yielded restrain-

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[2]The term "bacteria free filtrate" is used to refer to a filtrate free from bacteria in the usual form.

ing filtrates more often than mother cultures, attempts were made to produce slow butter cultures experimentally by exposure to the air. The method consisted of exposing to the air the milk from which butter cultures were made, or of bubbling air through the milk, and then inoculating the milk with normal butter cultures. The growth of certain butter cultures inoculated into milk given such treatment was sometimes restrained, thus indicating a relationship between the air which was in contact wtih the milk before inoculation, and the abnormally slow growth of the butter cultures inoculated into the milk. The suggestion that the inhibitory principle may come from the air is in agreement with the idea that it is a form of living matter, since various forms of life are present in the air.

PLASTICS FROM HIGH PENTOSANOCELLULOSIC MATERIAL[1]

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Since the development of a plastic molding compound from phenol and formaldehyde by Baekeland, the plastic field has grown rapidly. However, before plastics can be economically used to any great extent for large articles and in building construction the cost of manufacture must be greatly reduced. The work herein described has been directed toward the development of a cheap synthetic plastic from corncobs and oathulls.

HISTORICAL

Several early workers (1, 2, 4) have observed that cellulosic materials will condense with phenol in the presence of an acid catalyst to give resinous products. Work on a corncob-phenol plastic was started at Iowa State College when Moscrip (3) and Williams (5) attempted to use the furfural content of corncobs to produce a resin with phenol, in the corncobs and, thereby, obtain a plastic material. The plastic which they obtained gave considerable promise, and this work is a continuation of the research on plastics from these highly important cellulosic raw materials.

EXPERIMENTAL

The experimental work was divided into two sections. The first included the investigation of the nature of the reactions of the pure constituents of corncobs and oathulls with phenol and cresol. Purified cellulose, pentosans, pentoses and lignins were caused to react with phenol under the influence of sulfuric acid, zinc chloride, sodium hydroxide and hydrochloric acid as catalysts. In the reactions of cellulose and phenol, the proportion of cellulose to phenol was varied from 2:1 to 2:3 with a ratio of sulfuric acid catalyst to phenol of 1:5. The temperature of the reactions was varied from 90° to 160° C. The lignin, pentosans and pentoses were treated in a similar manner.

The second part of the experimental work was devoted to the development of a standard process for producing a plastic material, and to methods for improving this material. The process is as follows:

1. Five parts by weight of $ZnCl_2$ or H_2SO_4 are dissolved in an equal weight of water and then combined with 100 parts of cresol or phenol. The mixture is then heated to boiling in an autoclave.

2. Ground corncobs, 30 parts, or the same weight of oathulls are gradually introduced with stirring into the hot cresol mixture.

3. The autoclave is closed and the temperature kept at 140° C. for 3 hours.

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[1]Original thesis submitted June, 1934.

4. The pressure is then released and the reacted material is subjected to a vacuum distillation until the boiling point rises to 260-300° C., depending upon the desired melting point of the resin.

5. The resin is then run out into flat pans and cooled until solid.

6. The plastic molding compound is prepared by grinding together 40 parts of the powdered resin and 60 parts of a wood flour filler.

The effect of the stearic acid and zinc stearate on the plasticity of the molding material was studied by incorporating 2 per cent of either stearic acid or zinc stearate in the molding powder. The molding powder was also ground to pass a 200 mesh sieve to observe the effect of fineness on the plasticity.

The relation of molding temperature to strength of product was investigated by varying the molding temperature from 100° to 140° C. and testing the strength of the molded products on a Page impact testing machine.

A comparison was made of the effect, as fillers, of corncob flour, soybean meal, Sil-o-cel, calcium carbonate, fine and coarse asbestos, and wood flour on the water-resistance and strength of the molded products. Paraformaldehyde and hexamine were tried as hardening agents.

RESULTS

To sum up the results of the studies of the reactions of the cellulose, lignins, and pentosans with phenols, it was found that all would condense with the phenol in the presence of H_2SO_4, HCl or $ZnCl_2$ as catalysts. The product in each case was a black, tarry material which could be made into a rather brittle, black resin by removal of volatile constituents. Cellulose was also found to act in another way; it would dissolve in the phenol and zinc chloride, the latter acting as a solvent instead of a catalyst.

In the development of the plastic molding compound, both zinc stearate and stearic acid improved the plastic properties slightly, but gave the molded products a greasy appearing surface. Fine grinding increased plasticity but decreased strength. The optimum molding temperature for a molding powder containing 40 per cent resin and 60 per cent wood flour was found to be approximately 140° C. Wood flour and asbestos were by far the best fillers on the basis of strength and water-resistance tests. Molded products containing asbestos filler, though not quite as strong as those containing wood flour, were much superior in water resistance.

Hexamine was found to increase the strength of a molded disc so that it withstood the impact of a 2 Kg. weight by dropping 26 cm., whereas, the untreated disc withstood a drop of only 11 cm. The addition of paraformaldehyde did not increase the strength.

CONCLUSIONS

1. Cellulose, pentosans, pentoses and lignins can all be condensed with phenol to give resinous products.

2. A plastic material can be made from the combined solvent actions of zinc chloride and cresol; however, it has the disadvantage of not being very water-resistant.

3. The advantage of using $ZnCl_2$, as catalyst, over H_2SO_4 is due to the lower corrosive action of the plastic in the molding operation.

4. Asbestos and wood flour were proven to be the best fillers of those tested.

5. The additional plasticity derived from the use of zinc stearate or stearic acid does not warrant their use.

6. The addition of hexamine was decidedly beneficial as it reduced sticking in the mold and increased the strength.

SUMMARY

The reactions of the pure constituents of corncobs and oathulls were studied in an attempt to understand their influence in the corncob-cresol or oathull-cresol plastics.

Cellulose, lignins and pentosans were all found to condense with phenol in the presence of an acid catalyst to give similar resinous products.

A standard procedure was developed for preparing a molding compound from corncobs and cresol.

The properties of the molding compound were improved by a study of the effect of the molding temperature; by experiments with the use of plasticizers; by an investigation of various fillers, and through experiments on the effect of paraformaldehyde and hexamine as hardening agents.

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A STUDY OF CHLORINE STERILIZING COMPOUNDS

I. RELATIONSHIP BETWEEN pH AND OXIDATION POTENTIALS[1]

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The linear relationship between pH and oxidation potential has been shown to hold for Chloramine-T in all the concentrations used. The average slope was calculated to be —7.425. This linear relationship did not extend over the entire pH range. The limits through which the relationship was constant were determined by the concentration.

The oxidation potential, as displayed by active chlorine compounds, can only be considered as a measure of the intensity factor since the concentration of active chlorine appeared to have little effect upon the value of the potential developed. The oxidation potential was influenced by the presence of other salts in the menstrum.

The linear relationship for pH and oxidation potential can be extended to the inorganic calcium and sodium hypochlorates. The potential values for the inorganic hypochlorates were much larger than for Chloramine-T. A gradual change was noted in the oxidation potentials proceeding from 10 p.p.m. (active) to 500 p.p.m. (active). This change may be due to the large amount of inert material present. The inorganic hypochlorates having the general formula (OCl) m have been shown to have similar properties.

An explanation has been proposed, based on the electro-negativity of the (R) group, to account for the wide difference in the nature of the organic and inorganic hypochlorates.

Due to the fact that the pH and oxidation potential were a linear function for the compounds used, equations have been developed by which the pH or oxidation potential can be calculated.

The results have shown that the oxidation potential and the time to kill 99 per cent of the bacterial spores were a linear function between pH 6.0 and 7.0. A consideration of oxidation potential as a guide to germicidal efficiency has developed certain limitations. A mechanism has been offered to explain the manner in which the cells were destroyed by active chlorine.

It has been shown that the time to kill 99 per cent of the bacterial spores was a linear function of the percentage of active chlorine present as the undissociated hypochlorous acid. This would tend to show that the active germicide was the undissociated molecule.

The rate of decolorization of mercurochrome has been shown to be a linear function of the oxidation potential, and also a linear function of the percentage of HOCl present as the undissociated molecule.

Mathematically, the bleaching of mercurochrome and the time to kill 99 per cent of the bacterial spores were both linear functions of the same unit, namely, the oxidation potential, and therefore were linear in respect to each other. Thus, the bleaching of mercurochrome afforded a

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means of developing a suitable test for the germicidal efficiency of active chlorine compounds.

THE DETERMINATION OF THE EFFECT OF MANGANESE AND SULFUR ON THE MALLEABILIZATION OF WHITE CAST IRON[1]

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This study was undertaken in order to obtain more data concerning the effect of manganese and sulfur on the rates of malleabilization or graphitization of white cast iron. Two basic compositions of white iron were chosen. The composition of one series was approximately 2.40 per cent carbon, 1.0 per cent silicon, 0.040 per cent sulfur, 0.15 per cent phosphorus, and manganese, varying from 0.006 per cent to 0.37 per cent. The composition of the second series was approximately: 2.10 per cent carbon, 1.12 per cent silicon, 0.040 per cent sulfur, 0.15 per cent phosphorus and manganese varying from 0.006 to 0.0397 per cent.

The malleabilization was carried out in two so-called "stages" of temperature. The first stage, or the high temperature, was 926° C. (177° F.) and the second stage, or low temperature, was 704° C. (1300° F.).

EXPERIMENTAL PROCEDURE AND DATA

The materials used in the preparation of these alloys consisted of Armco iron, graphite, ferro-silicon (46 per cent silicon), ferro-manganese (46 per cent manganese), ferro-phosphorus (25 per cent phosphorus), and ferrous sulfide (50 per cent sulfur).

About 5,000 grams of Armco iron were melted in a small Plumbago crucible by means of a 35 kv-a Ajax Northrug electric furnace. The graphite, ferro-silicon, ferro-phosphorus, and ferrous sulfide were then added in quantities to give the desired white iron compositions. Certain amounts of this were then remelted and ferro-manganese added to produce the desired percentage of manganese. The final bars poured were cast in individual sand moulds, in 12 inch lengths having five-eights inch diameter. A pouring temperature of 1316-1371° C. (2400-2500° F.) was used as determined by a Leeds and Northrup optical pyrometer. Before pouring, the sand moulds had been at room temperature for 48-50 hours and after pouring were broken after an interval of 30 minutes.

The analytical determinations were made by the following methods. The carbon was determined by direct combustion in oxygen with ascarite as the absorbent. The silicon method used was that of nitro-sulfuric acid dehydration. The manganese was determined by the sodium bismuthate method. The barium sulfate precipitation method was used for sulfur; and for phosphorus, the alkali-acid titration method.

Samples of about one to two inches in length of the various alloys were placed in a Hump annealing furnace which had previously been brought to a temperature of 926° C. (1700° F.). To avoid decarburization the samples were packed with graphite in small iron or carbon con-

tainers, but in spite of this precaution slight decarburization occurred at the surface.

Iron-constantan thermocouples were used and the temperature controlled and recorded by a Leeds and Northrup recorder. This temperature was frequently checked by a portable pyrometer indicator which in turn had been checked by a Leeds and Northrup Student Potentiometer (new model) and found satisfactory. The rate of malleabilization was determined by taking samples from the annealing furnace at definite intervals of time, with subsequent microscopic examination. The absence of all free cementite constituted the first stage.

Upon completion of the first stage of malleabilization at 926° C. (1700° F.), the samples were again placed in containers and packed with graphite. The containers with the samples were then placed in the furnace and heated to 926° C. (1700° F.), whereupon the power was turned off and the samples allowed to cool in the furnace in the second stage temperature, 704° C. (1300° F.). The rate of malleabilization of the second stage was again determined by withdrawing samples at definite time intervals. These samples were then examined microscopically for complete removal of pearlite.

In tables 1 and 2 the most representative values of this study are tabulated.

TABLE 1

No.	Mn	S	Mn–2S	Mn/S	Hours at 925° C.	Hours at 704° C.	Total hours
26	0.061	0.038	—0.015		9	80	89
31	0.087	0.043	0.001	2.0-1	9	80	89
32	0.098	0.043	0.012	2.2-1	24	30	54
69	0.171	0.0201	0.131	8.5-1	7	40	47
43	0.155	0.045	0.065	3.4-1	12	30	42
40	0.149	0.040	0.069	3.7-1	7	25	32
42	0.156	0.040	0.076	3.9-1	8	25	33
45	0.188	0.056	0.076	3.9-1	6	30	36
68	0.180	0.033	0.114	5.4-1	8	25	33
44	0.218	0.035	0.148	6.2-1	6	25	31
55	0.220	0.037	0.146	6.0-1	6	30	36
74	0.350	0.040	0.270	8.8-1	6	30	36
73	0.358	0.041	0.276	8.7-1	6	35	41
75	0.361	0.040	0.281	9.0-1	6	35	41
66	0.385	0.035	0.315	11.0-1	8	30	38
67	0.397	0.038	0.321	10.4-1	5	40	45
65	0.397	0.036	0.325	11.0-1	5	40	45
64	0.388	0.038	0.312	10.2-1	12	40	52

DISCUSSION OF RESULTS

In plotting the total time of malleabilization as ordinate against the Mn, S ratio as abscissa, it was observed that the minimum for the 2.10 per cent carbon series was 4.6 and that the minimum for the 2.40 per cent carbon series was 5.4. In a comparison of the Mn-2S values to the total time of malleabilization, it was noted that for samples 44, 55, and 68, the

TABLE 2

No.	Mn	S	Mn-2S	Mn/S	Hours at 926° C.	Hours at 704° C.	Total hours
20	0.083	0.051	—0.019	1.6-1	20	35	55
21	0.079	0.042	—0.005	1.9-1	30	30	60
38	0.129	0.054	0.021	2.4-1	20	35	55
29	0.084	0.023	0.038	3.7-1	9	40	49
27	0.089	0.020	0.049	4.4-1	6	30	36
28	0.098	0.023	0.052	4.3-1	7	35	42
33	0.120	0.056	0.008	2.1-1	7	34½	41½
35	0.111	0.060	—0.009	1.9-1	7	15	22
36	0.126	0.053	0.020	2.3-1	7	18	25
72	0.247	0.049	0.149	5.0-1	7	22½	29½
71	0.244	0.042	0.160	5.8-1	6	22	28
70	0.254	0.040	0.174	6.4-1	7	22	29
50	0.273	0.031	0.211	8.8-1	7	25	32
47	0.274	0.042	0.190	6.5-1	12	25	37
48	0.271	0.028	0.215	9.7-1	6	30	36
49	0.270	0.033	0.204	8.2-1	7	30	37
53	0.337	0.040	0.257	8.4-1	7	40	47
54	0.356	0.040	0.276	8.9-1	8	30	38
52	0.372	0.033	0.306	11.6-1	7	30	37
51	0.374	0.032	0.310	11.7-1	12	30	42

Mn-2S was 0.136 and the total time 33½ hours. These values were the average of the three. In the case of samples 70, 71, and 72, the average Mn-2S was 0.161 and the total time 28.8 hours. The values for the Mn, S ratios are in fair agreement with those of Yemenidjian (1) and those of Kikuta (2).

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THE ROLE OF INORGANIC SUBSTANCES AND AMINO ACIDS IN THE REGENERATION OF HEMOGLOBIN IN THE RAT[1]

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There has been considerable controversy as to whether other substances, both inorganic and organic, would replace copper as a hemotinic, since copper was found to act as a supplement to iron in the diet for the relief of nutritional anemia in rats (1). Investigators from several different laboratories have reported hemoglobin regeneration due to iron salts alone (2) in rats made anemic by an exclusive milk diet. Beard, Baker, and Myers (3) stated that the supplementation of a milk and iron ration with salts of manganese, arsenic, titanium, zinc, rubidium, chromium, selenium, mercury, vanadium, and cobalt also formed hemoglobin. Other workers observed a distinct hematopoietic action by feeding tryptophane, histidine, arginine, tyrosine, and glutamic acid along with iron to anemic animals (4) (5).

The purpose of this investigation was to find out whether or not hemoglobin could be synthesized in anemic rats, maintained upon a milk and iron diet, by the administration of various inorganic substances and amino acids. Other studies were made upon the daily copper and iron requirements of anemic rats and also the effect of insoluble copper and iron compounds upon nutritional anemia.

EXPERIMENTAL

The experimental animals were rendered anemic by feeding a milk diet. They were housed individually in galvanized iron wire cages and fed a basal diet of milk collected specially in glass in order to insure a food of low copper content. The inorganic materials investigated were tested for copper by means of spark spectograms. Precautions were taken in all experiments to eliminate copper contaminations which might have crept in due to faulty feeding and housing of the animals.

All rats were bled weekly from the tail and the percentage of hemoglobin determined by the Newcomer acid hematin method. Records of both growth and hemoglobin values were used to determine whether or not a substance was curative in nutritional anemia.

CONCLUSIONS

Pure iron salts, fed in low-copper milk, will not regenerate hemoglobin in anemic rats.

Inorganic compounds of titanium, manganese, vanadium, arsenic, germanium, zinc, chromium, tin, mercury, cobalt, silver, and gold do not act as hemotinics when fed along with iron as ferric chloride in low-copper milk.

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[1]Original thesis submitted July, 1933.

Intraperitoneally injected inorganic compounds of nickel, zinc, germanium, manganese, vanadium, arsenic, titanium, selenium, mercury, rubidium, and chromium will not cause hemoglobin formation in anemic animals fed upon a low-copper milk and iron ration.

The addition of tyrosine, tryptophane, arginine, glutamic acid or aspartic acid to a low-copper milk and iron diet fails in hematopoiesis.

Anemic rats may be cured by intraperitoneal injections of 0.002 mg. of copper as copper sulfate along with 0.1 mg. of iron as colloidal ferric hydroxide administered daily.

Insoluble copper compounds such as cupric sulfide, cuprous oxide, and cuprous iodide are utilized by anemic animals for hemoglobin regeneration.

Intraperitoneal injections of iron alone, either as the chloride or citrate, will give a temporary relief to rats suffering with nutritional anemia.

Colloidal solutions of cupric hydroxide and ferric hydroxide administered simultaneously either orally or intraperitoneally will cure nutritional anemia in rats.

Iron in the form of ferric hydroxide suspension, when fed along with copper as copper sulfate, restores the hemoglobin level to normal.

Weak solutions of hydrochloric acid injected intraperitoneally into anemic rats give temporary relief.

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THE BIOCHEMISTRY OF THE PRODUCTION OF 2,3-BUTYLENE GLYCOL BY FERMENTATION[1]

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One of the fields of research now occupying the attention of many agencies is the utilization of agricultural products, including the so-called farm wastes, as raw materials of the chemical industries. Among the many products formed by the action of micro-organisms upon the carbohydrates, the chemical 2,3-butylene gylcol offers many possibilities as a useful industrial chemical. The production of this chemical by fermentation has been studied for several years in these laboratories. The object of this thesis was to extend these preliminary findings and to ascertain the conditions leading to the highest possible yield of 2,3-butylene glycol in a medium of known composition. The substrate employed was sucrose.

Most of the experimental work was done with the organism *Aerobacter pectinovorum.* After the optimum medium had been developed, the following organisms were tested: two strains of *A. cloacae,* two of *A. aerogenes* and one of *A. pectinovorum.* There was no significant difference in the yield of 2,3-butylene glycol produced by these different organisms of the genus Aerobacter.

The following factors were studied as to their effect upon the yield of 2,3-butylene glycol: pH; concentration of sucrose; concentration of magnesium sulfate; concentration of ammonium chloride; concentration of secondary potassium phosphate and concentration of calcium chloride. The general procedure was to vary one factor at a time, the others being held constant.

Each fermenting medium was adjusted daily to a definite pH by the addition of 1 M sodium carbonate solution under sterile conditions. The total acidity developed was calculated from the total amount of the carbonate added. The fermenting media were also analyzed for unfermented sugar and for the 2,3-butylene glycol produced. The sugar was determined by the Shaffer and Hartmann method. The method of analysis for the 2,3-butylene glycol was developed during the course of the research and consisted briefly in the following procedure:

To each flask, after fermentation was completed, as evidenced by the cessation of the formation of acid, was added 1½ cc. of 12 N sodium hydroxide. The alkali caused a precipitation of suspended material, including bacteria, leaving a clear solution for analysis. A 20 cc. portion of the clear supernatant liquid was placed, together with 21 grams of powdered potassium carbonate, in a glass extraction tube which was so constructed that it, together with a small funnel, could be suspended from an A. S. T. M. extraction apparatus, and hence was adapted for the continuous extraction of liquids with an immiscible solvent. Stirring the mixture until all the salt was dissolved gave approximately 26 cc. of a saturated potassium carbonate solution.

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[1]Original thesis submitted June, 1934.

The temperature of the water bath was so regulated that about 2 drops of ether condensed each second (45-50° C.), and the extraction was continued for 5 days at this rate. This prolonged extraction was found advisable for complete removal of the glycol. The ether was then evaporated at 45-50° C. and the flask allowed to stand un-stoppered until attaining constant weight (about 15 hours). The amount of impurities in the glycol separated by this method is quite small, as was found when this fraction from a large amount of fermentation mixture was examined.

By the use of some carefully fractionated and remarkably pure glycol (B.P. 182.5° C. corrected), data were obtained on the readings of a dipping refractometer in various concentrations of the glycol in water, and it was found that the refractometer reading is a linear function of the concentration of the glycol. In order to check the gravimetric method, 20 cc. of water were added to the weighed residues from the extractions, and the solutions were analyzed by the refractometric method. In general, the refractometric method gives somewhat lower results than those obtained by weighing. In the data presented in this thesis ,the yields of glycol represent the average of the values obtained by the two procedures.

The results of these experiments show that in an inorganic medium, for the maximum conversion of sucrose into 2,3-butylene glycol:

1.   There is a definite optimum pH of about 6.2.

2.   The most efficient conversion of the sugar occurs at a concentration of 8 per cent.

3.   There is a definite optimum concentration of magnesium sulfate at 0.175 per cent.

4.   Ammonium chloride is very essential and at least 0.3 per cent must be present. A concentration higher than 0.3 per cent does not give yields of glycol differing appreciably from the yield at 0.3 per cent.

5.   There is a definite optimum concentration of secondary potassium phosphate at 0.175 per cent.

6.   A trace of calcium chloride is essential, but any appreciable concentration is somewhat harmful; a 0.1 per cent concentration being slightly more harmful than a 1.0 per cent concentration.

7.   Various species of the genus Aerobacter produce approximately the same yields of glycol under like conditions.

8.   Under optimum conditions the yield of glycol amounts to about 50 per cent, by weight, of the sucrose fermented.

THE EFFECT OF CERTAIN BACTERIA ON THE RIPENING OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK[1]

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In many parts of the United States the milk delivered to cheese factories is frequently of poor quality. Such milk may contain various types of microorganisms which not only cause undesirable flavors and textures in the cheese, but which are objectionable from the standpoint of public health. Pasteurization of milk of poor quality is a logical procedure to insure cheese of at least fair quality. Experience has shown, however, that cheddar cheese made from pasteurized milk rarely develops the full, characteristic flavor normally found in raw milk cheese of good quality. The pasteurized milk cheese, in addition to its usual lack of flavor, generally requires an extended ripening period to insure a normal degree of protein breakdown.

The destructive effect of pasteurization on certain of the bacteria necessary for bringing about normal cheese ripening probably accounts in part for the undesirable characteristics of pasteurized milk cheddar cheese. It is likely, therefore, that the addition of certain strains of bacteria to pasteurized milk used for cheesemaking would produce desirable chemical changes in the cheese with a corresponding improvement in flavor. The addition of special bacterial cultures in this manner also opens up many possibilities in connection with the development of new and desirable flavors in cheese of the cheddar type.

The effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk was studied with milk obtained from three dairies. The milk was inoculated with varying numbers of the test bacteria previous to the cheesemaking process, and the cheese was examined for the nitrogen distribution and the flavor at regular intervals during ripening. The bacteria used included several strains of *Lactobacillus casei*, and one strain each of *Aerobacter oxytocum, Streptococcus liquefaciens, Streptococcus paracitrovorus*, and an unidentified Micrococcus. Experiments were also carried out to determine the effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese. For making the cheese, 40-gallon steam-jacketed cheese vats were used, and longhorn cheese weighing approximately 12 pounds each were made.

Thirteen series of cheese were manufactured; each series contained three cheese manufactured at the same time from equal portions of a single lot of milk. Usually one cheese was made from raw milk, one from pasteurized milk, and one from pasteurized milk plus a milk culture of a test organism, or 10 per cent raw milk. In some cases, however, all three of the cheese in a series were made from pasteurized milk, and each of two portions of milk was inoculated with a milk culture of a test organism.

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[1]Original thesis submitted June, 1934.

Cheese juice, for analytical purposes, was obtained directly from the cheese by submitting mixtures of finely divided cheese and sand to relatively high pressures. To extract the juice from cheddar cheese, 400 grams of cheese were first cut into thin shreds with a small soap grater. The shreds thus obtained were mixed by hand with 800 grams of fine sea sand. A hydraulic laboratory press with an iron cylinder attachment was used to press the juice from the cheese. For each extraction the hollow iron cylinder was entirely covered on the inside with a closely woven linen cloth, and the mixture of cheese and sand placed into the cylinder between felt filter pads. The cylinder was set on an iron plate at the base of the press, and as the pressure was slowly applied, the cheese liquid was forced out of the cylinder, through clearance spaces, on to a grooved outlet around the outer edge of the plate; from here it dropped into a beaker.

Changes in the nitrogen distribution in cheese were determined by chemical analyses of cheese juice after approximately one, five, ten and fifteen weeks of ripening at about 4° C. The chemical analyses included determinations of total nitrogen, amino nitrogen and various proteins and protein decomposition products soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid and tungstic acid. The results obtained were expressed as the cubic centimeters of 0.1 normal acid equivalent to the nitrogen in 1 cc. of cheese juice. The cheese was scored for flavor at the same periods that chemical analyses were made.

A steady breaking down of the proteins during the ripening was shown by increases in all the nitrogen fractions determined. Cheese made from raw milk and cheese made from pasteurized milk inoculated with test bacteria or raw milk showed a more rapid and extensive breakdown than the control pasteurized milk cheese. Practically all of the control pasteurized milk cheese was characterized by having a flat flavor and a tough, rubbery body.

Four of the seven strains of *L. casei*, when added individually to pasteurized milk, appeared to improve the flavor and hasten the ripening of the resulting cheese. Two of the strains consistently produced a distinctly pleasing, buttery flavor which was very desirable. The addition of a strain of *S. liquefaciens* in small numbers to pasteurized milk produced well ripened cheese in a comparatively short ripening period, while the addition of relatively large numbers of these bacteria produced extremely bitter and soft-bodied cheese, the juice of which contained relatively large amounts of nitrogen soluble in trichloracetic acid and ethyl alcohol, but insoluble in phosphotungstic acid and tungstic acid.

When a culture of *A. oxytocum* was added to pasteurized milk, the resulting cheese had an unclean flavor and the juice contained relatively large amounts of nitrogen insoluble in trichloracetic acid. The addition of *S. paracitrovorus* or the Micrococcus appeared to slightly improve the flavor and hasten the protein breakdown of pasteurized milk cheese. The addition of 10 per cent raw milk to pasteurized milk produced cheese very similar to raw milk cheese in nitrogen distribution and flavor.

The juices of the cheese made from raw milk, the cheese made from pasteurized milk after the addition of desirable bacteria, were all characterized by the presence of large amounts and percentages of nitrogenous fractions which were soluble in trichloracetic acid but insoluble in ethyl

alcohol, and also by the presence of small amounts and percentages of nitrogenous fractions which were insoluble in trichloracetic acid, as compared to the juices of the control cheese made from pasteurized milk. It appears that the formation, during the ripening, of relatively large amounts of a compound or compounds soluble in trichloracetic acid and insoluble in ethyl alcohol, may be responsible in part for the characteristic flavor of high quality cheddar cheese.

PHYSIOLOGICAL AND TOXICOLOGICAL STUDIES ON INSECTS[1]

Edward Rawson McGovran

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PART I. RESPIRATORY RESPONSES OF ADULT ORTHOPTERA TO CERTAIN GASES

A method of measuring the tracheal ventilation in insects is described and illustrated by a figure. The apparatus consists of two closed chambers, one enclosing the head and thorax of the insect and the other the abdomen. The insect is sealed in between the chambers so that air can only pass from one chamber to the other by being forced through the tracheal system of the insect. As the pressure is kept equal to atmospheric pressure at all times in both chambers, any air movement from one chamber into the other must have been produced by the breathing movements of the insect. The pressure and volume in each side of the apparatus is controlled by reservoirs in which the water level can be readily adjusted and horizontal capillary tubes which are closed by short columns of water. The water in the capillary tubes is free to travel in either direction along the capillary tubes and is so easily moved that it responds immediately to any movements of air produced by the insect in ventilating its tracheal system.

Adula female *Chortophaga viridifasciata* DeG., *Arphia sulphurea* Fab., *Dissosteira carolina* Linne, *Melanoplus bivittatus* Say, *Melanoplus differentialis* Thomas, and *Hippiscus*, species undetermined, were studied.

The respiratory movements of the insects produced a streaming movement of air through the tracheal system. The air was inhaled principally into the thorax and exhaled principally from the abdomen.

Adult female *C. viridifasciata* at 28° C. passed an average of 0.22 cc. of air through their tracheal system per minute per gram of body weight with a minimum of 0.12 cc. and a maximum of 0.33 cc.

Adult female *C. viridifasciata* exhaled an average of 20 per cent of the total CO_2 evolved from the thorax and 80 per cent from the abdomen at 23° C. If it can be assumed that all the air exhaled contains the same percentage of CO_2, it is evident that only part (about 80 per cent) of the air movement within the trachael system of these insects is a through movement in the direction given above.

In 93 per cent of the tests 15 per cent CO_2 produced an increase in the rate of tracheal ventilation. The maximum increase for any period was slightly more than 2,000 per cent. Seventy-two per cent of the tests showed a reversal of the direction of air movement through the tracheal system.

One per cent CO_2 did not consistently increase the rate of tracheal ventilation nor reverse the direction of air movement through the tracheal system in a single instance.

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[1] Original thesis submitted December, 1933.

Sub-lethal concentrations of CS_2 and nicotine vapor usually increased the rate of trachael ventilation.

High concentrations (0.2 per cent) of HCN produced a rapid fall in the rate of trachael ventilation.

Concentrations of CS_2 and HCN which killed the insect slowly produced an increase in the rate of tracheal ventilation followed by a decrease as the gas rendered the insect less and less active.

The apparatus as operated in these tests recorded only the movement of air completely through the tracheal system. In many of the tests, especially with 15 per cent CO_2, the insects inhaled and exhaled rapidly and deeply, but did not produce a correspondingly large movement of air into the thorax and out of the abdomen. This was most noticeable when the direction of air movement through the tracheal system was being reversed. First, the air movement in the normal direction would be reduced until it stopped entirely, but air was still rapidly inhaled and exhaled with each spiracle apparently performing equally as inhalatory and exhalatory orifices. After a short period of this type of breathing more air began to be exhaled from the thorax than was inhaled into the thorax, which was a reversal of the normal.

Nine references are cited.

PART II. TOXICITY OF PETROLEUM OIL MIXED WITH CERTAIN CHEMICAL COMPOUNDS TO LARVAE OF CARPOCAPSA POMONELLA LINNE

Young larvae of *Carpocapsa pomonella* Linne were placed on apples that had been sprayed with unemulsified petroleum white oil to which had been added some material to increase its toxicity to the larvae. The number of blemishes produced on the fruit by the larvae was then recorded.

The following materials were added to the white oil: tannic acid, nicotine, nicotine sulphate, goulac, 1-3-8 trinitronaphthalene, methyl salicylate, para-dibromobenzene, naphthalene, copper cyanide, copper oleate, ground pyrethrum, alpha-naphthylamine, iodine, beta-iodo-naphthalene, meta-dinitrobenzene, dibromo-ortho-cresol, para-chloroaniline, para-nitroiodobenzene, 3-5 dibromo-ortho-cresol, xylene, allyl-isothiocyanate, beta-chloronaphthalene, carbon tetrachloride, cetyl arsenite, piperine, toluene, ortho-toluidine, thymol, ortho-dichlorobenzene, fluorobenzene, barium stearate, aniline, tetra-chloroethylene, 2-4 dichloro-6-phenyl-phenol, alpha-nitronaphthalene, para-nitrochlorobenzeen, 2-5 dichloro-aniline, 1-2-4-5 tetrachlorobenzene, monochloronaphthalene, copper sulfocyanide, rotenone, arsenious oxide, ortho-nitrochlorobenzene, barium oleate, benzene, chloroform, 1-nitro-2-naphthol, lamp black and sodium arsenite, benzyl arsenic acid, thallous malonate, sodium cyanide, meta-dichlorobenzene, phenol, trinitro-resorcinol, ortho-phenyl-phenol, 9-10-dichloroanthracene, trihexachloronaphthalene, menthol, para-dichlorobenzene, sodium sulfocyanide, dinitro-phenol, sodium-ortho-phenyl-phenylate, phenyl-iso-thio-cyanate, triphenyl-arsine, beta-bromonaphthalene, dichloronitrobenzene, para-para-dichlorodiphenol, ortho-cresol, beta-naphthol, 2-chloro-6-phenyl-phenol, 3-5-dinitro-ortho-cresol, chloropicrin, bromopicrin, bromine, chlorine, nitrobenzene, pyridine, sodium-chloro-ortho-cyclohexyl-phenate, para-dimethylaminobenzylaldehyde, cyanamide, bromoform, 1-2-4- trichlorobenzene, dinitronaphthalene, phenyl-

alpha-naphthylamine, ortho-cyclo-hexylphenol, phenacyl chloride, diphenylaminoarsine, ground red pepper, anthracene, cumidine, chloroacetone, 1-3-5 trinitrobenzene, 2-4-dichlorophenol, beta-chloroethyl-para-toluene sulfonate, bromo-beta-naphthol, cetyl fluoride, carbon disulfide, iodoform, sodium fluoride, 2-4 dinitrochlorobenzene, cyanogen bromide, 2-4-dinitrobromobenzene, cetyl alcohol, metallic arsenic, para-bromobenzonitrile, beta-naphthylamine, para-nitro-bromobenzene and para-toluidine. These materials were tested in various mixtures in the white oil. The compounds are listed roughly in the order of their toxicity to the larvae. The concentration of the material in the oil varied from 1 per cent to 25 per cent.

Of the mixtures tested, nicotine, nicotine sulphate, 1-3-8 tri-nitronaphthalene, methyl salicylate, copper cyanide, copper oleate, alphanaphthylamine and iodine were the most toxic at concentrations of 2 per cent or less in white oil. Of these materials, nicotine and nicotine sulphate mixtures were the only ones that gave more than 71 per cent control.

Thirty-six series of compounds were studied. Plant extract mixtures were the most toxic. Nicotine was the most toxic material tested in this series.

The copper mixtures tested possessed marked toxicity to the larvae. Copper cyanide was the most toxic material.

Barium, nitro-naphthalene and hydroxyl bromine mixtures and beta-iodo-naphthalene ranked next in toxicity. Barium stearate was the most toxic barium compound tested. 3-5 di-bromo-ortho-cresol was the most toxic at low concentrations of the bromine compounds.

Iodine, chlorine and bromine and some of their compounds ranked in the order listed in toxicity to codling moth larvae in this series of tests.

Derivatives of naphthalene and naphthalene mixtures were slightly more toxic than derivatives of benzene and benzene mixtures in general.

Anthracene and 9-10 dichloroanthracene were practicaly non-toxic to codling moth larvae.

Aromatic compounds were more toxic than aliphatic compounds.

Cetyl arsenite was more toxic than the arsines or inorganic arsenic mixtures that were tested.

Thirty-five references are cited.

A STUDY OF THE CORYNEBACTERIA (DIPHTHEROIDS) ASSOCIATED WITH DISEASES OF DOMESTIC ANIMALS[1]

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During recent years many contributions have been made concerning the relationship of diphtheroid bacteria to animal diseases, but until the time of this study the various species had not been correlated satisfactorily. In order to secure an adequate relationship of the different species of Corynebacteria associated with animal diseases, the literature was completely reviewed and 49 strains of Corynebacteria were examined. The study of these 49 cultures was restricted to morphology, staining reactions, cultural characteristics, biologic reactions, fermentation and agglutination. On the basis of the characteristics of the cultures, 9 were found to be *Corynebacterium pyogenes*, 12 were *Corynebacterium pseudotuberculosis*, 20 were *Corynebacterium renalis* and one was *Corynebacterium equi*. Two cultures isolated from milk were not included with any of the above four species, and two non-chromogenic, rapidly-growing strains also were placed in a separate type. Three cultures which appeared to be variants were considered to be different than the others, although they originated from *Corynebacterium pseudotuberculosis* and *Corynebacterium renalis*.

Photographs showing the morphology of the four species mentioned above and also their colony characteristics are to be found in the original thesis.

The most distinctive characteristics of *Corynebacterium pyogenes* were found to be: morphology; aerobic as well as anaerobic growth; reproduction only in media containing blood, blood serum or milk; coagulation of milk with subsequent digestion of the curd; liquefaction of gelatin and coagulated blood serum; production of acid in dextrose, levulose, galactose, mannose, sucrose, lactose, maltose and dextrin and by some strains, xylose.

Corynebacterium pseudotuberculosis was characterized by its dry granular colonies; pellicle formation on fluid media; inability to alter milk, gelatin and coagulated blood serum. It produced acid in dextrose, levulose, mannose, sucrose and maltose.

It was found that *Corynebacterium renalis* grew in moist isolated colonies, digested milk casein producing an alkaline reaction, but had no action on gelatin or solidified blood serum. It fermented dextrose and some strains also fermented levulose and mannose.

Corynebacterium equi was recognized by its ability to produce a red-colored pigment, its profuse viscid growth, reduction of nitrates and inability to ferment carbohydrates.

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[1]Original thesis submitted July, 1933.

None of the four species demonstrated a close serologic relationship except *Corynebacterium pseudotuberculosis* and *Corynebacterium renalis*. These two species showed marked cross agglutination.

Orange colored variants were isolated from three cultures. These variants resembled their parent cultures in morphology but were smooth and pigmented and failed to ferment carbohydrates, whereas the parent cultures were usually rough, cream colored and demonstrated some fermenting ability.

CLASSIFICATION

It was found that all four of the species had been named correctly by previous investigators. *Corynebacterium pyogenes* was first called *Bacillus pyogenes* by Glage, but Eberson gave it its present name in 1918. The correct designation for the organism was considered to be *Corynebacterium pyogenes* (Glage) Eberson.

Corynebacterium pseudotuberculosis was first named, in 1894, by Preisz, *Bacillus pseudotuberculosis ovis*. In 1918, Eberson gave it the name *Corynebacterium pseudotuberculosis*. In was believed that Bergey et al used an incorrect name in 1923, 1925 and 1930 when they proposed *Corynebacterium ovis*.

The name, *Corynebacterium renalis*, has not appeared in much of the recent literature even though Jones and Little, in 1926, described the organism quite adequately without making reference to its name. Enderlen described the organism in 1891, giving it the name *Bacillus renalis bovis*. The same year Höflich also described it and proposed the name *Bacillus pyelonephritidis boum*. Bollinger, however, made a report in 1890 upon the work of Enderlen and suggested the name *Bacillus renalis bovis*. Ernst gave the organism the name *Corynebacterium renalis* in 1906. Since that time, Ford, in 1927, has been the only writer to consider the organism a distinct species.

Magnusson named *Corynebacterium equi* in 1923. Lutje named it *Corynebacterium pyogenes (equi) roseum* in 1924. He evidently was not aware of the previous description given by Magnusson. The organism has received rather complete description in the United States by Dimock and Edwards.

The type of variation encountered in the study was of great interest, because of the possible relationship of the variants to the organisms isolated by Daines and Austin from the skin lesions of tuberculin reacting cattle.

I. SOME FACTORS AFFECTING THE PRODUCTION OF INSULATION BOARD

II. THE DEVELOPMENT OF THE COMMERCIAL PRODUCTION OF REFRIGERATION BOARD AND PRESSBOARD[1]

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The problem was undertaken to further the production of synthetic lumber from cornstalks. The problem was divided into: first, factors affecting the production of insulation board, and second, the development of refrigeration board and pressboard.

EXPERIMENTAL

PART I

The first step of the investigation was to analyze the commercial boards for rosin, and the percentage of ash. The percentage of rosin was determined in each board. The percentage of ash was secured and the ash was analyzed for SiO_2, Fe_2O_3, and Al_2O_3. The boards were then subjected to various humidity conditions in order to study their respective expansion and contraction. Samples of each board were measured both before and after being exposed to the following: various humidities; drying to constant weight; immersion in water; and change in temperature at various humidities. Sodium silicate, ammonium chloride, ammonium acid phosphate, ammonium fluoride, ferrous sulphate, and various potassium salts were each added to cornstalk pulp as a possible fireproofing agent. A commercial fireproofing salt was also used in this work. Samples of commercial wall board were subjected to conditions suitable for mold growth. Various chemicals were added to the cornstalk pulp in order to inhibit mold growth.

EXPERIMENTAL

PART II

(A)

Various refining methods were used to produce a suitable refrigeration board from cornstalks. Boards were made from the various physical constituents of the corn plant. A suitable method for extracting the pith from the cornstalk was developed. A beater equipped with a revolving screened cylinder removed the pith from the beater. The pith was refined lightly in a jordan and then formed into boards and dried without pressure. The pithboard required a long time to dry since large amounts of water were retained. Such drying methods as: air drying; steam oven drying; vacuum drying; forced air drying; and electric drying were in-

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vestigated. An apparatus was built in order to test the insulating value of pithboard.

<center>(B)</center>

The production of a hard, dense pressboard was undertaken. Such relationships as: beating time to strength; fiber length to strength; rod-mill and claflin refining to strength; and freeness to strength were determined. Different methods of drying the wet mat before pressing were employed. The effect of various pressures; length of time of pressing; and temperature of pressing, on strength, were secured. Various sizing materials were used for sizing pressboard. Each of the following sizing materials; rosin and alum; paraffin and alum; sodium alginate; paraffin emulsions; and asphalt emulsions, were investigated. Parawax, paraffin oil, Halowax and Bakelite varnish were used as possible surface sizes.

<center>CONCLUSIONS</center>

<center>PART I</center>

NuWood and Insulite contained more rosin than any of the other commercial boards, while Celotex and cooked Maizewood contained the least. Insulite seemed to be waterproofed better than the rest of the boards. NuWood was the poorest sized board. Masonite yielded the lowest ash, while Maizewood yielded the highest. Boards made from wood showed less ash than boards made from other fibrous materials. The commercial boards, which yielded high ash, also yielded high silica. The ash from cooked Maizewood proved to be 75.8 per cent silica. The boards made from materials other than wood seemed to contain less aluminum oxide than those made from wood. Insulite was twice as strong as the other boards. The majority of the boards tested around three hundred and fifty pounds to the square inch. Masonite seemed to expand more, both in length and in width, while Maizewood expanded the least when placed at laboratory conditions. Celotex showed the least amount of expansion in length and width both at the one hundred and the seventy per cent humidity. Little difference was noticed in the expansion of the unsanded Maizewood and the sanded Maizewood boards. No appreciable change in length or width was noticed on finished boards during the first hour after they were removed from a constant temperature oven. The boards attained their original length and width after standing twenty-four hours at a temperature of 80° F. and thirty per cent humidity. Maizewood lath expanded 0.78 per cent in length and 0.69 per cent in width when immersed in water for twenty-four hours. The lath contracted 0.38 per cent in length and 1.00 per cent in width after drying five days at 80° F. and thirty per cent humidity. Boards containing from four to six per cent moisture seemed to expand and contract less when removed from the dryer than did boards containing a higher or lower percentage of moisture. Boards containing high percentages of newsprint expanded the same amount as boards containing less newsprint. Temperature seemed to play a very little part in expansion at each humidity. Maizewood expanded more in length than any other commercial board. No difference was noted in width. Boards could not be fireproofed by adding different amounts of sodium silicate to the pulp in the beater.

Boards could be fireproofed by applying ammonium phosphate and other chemicals to the surface. Maizewood was fireproofed one hundred per cent by adding a commercial fireproofing compound to the pulp in the mixing tanks. The boards were not waterproofed sufficiently after the treatment was completed. Commercial wall boards molded if they were placed in a warm, moist atmosphere, but not before fifty to sixty days. The edges showed signs of molding before the surfaces. Boards treated with zinc chloride, copper sulphate, and mercuric chloride did not mold for some weeks.

<center>PART II</center>

The whole stalk furnished a fair grade of refrigeration board, but not nearly as good as the pith alone. A very good grade of pith could be separated from the outer fiber by the flotation method. Eighty per cent of the pith could be removed without much cortex adhering. Refrigeration board could not be dried in air commercially, due to the long time required for drying. Boards dried in the steam oven were of good quality. This method of drying was also expensive, for the efficiency of the dryer was only 24.9 per cent. Vacuum drying increased the efficiency somewhat, but it still was too expensive a process. Forced air drying seemed to give the best quality boards even though they did dry a little slower than any of the rest. Electricity proved to be very expensive for drying pithboard.

Cooking improved both the appearance and strength of the pressboard. Much time was saved by refining the pulp in a rodmill and claflin. The strongest boards were produced from pulp containing 66 per cent or more moisture. A wet mat still contained 54.5 per cent moisture even though it was pressed at eight hundred and seventy-four pounds per square inch. Thirty minutes was a sufficient length of time for pressing boards of one-eighth inch thickness. The strength of boards pressed at four hundred pounds per square inch was very little different from the strength of those boards pressed at one hundred and forty pounds. A high percentage of size weakened the strength of the pressboard. Boards were waterproofed by means of paraffin emulsions. Sodium alginate proved to be a very poor sizing material. Asphalt and pitch did not waterproof the board as well as paraffin. The strength was decreased in every case where any of three sizes were used. Surface sizing proved to be almost worthless. A Bakelite varnish coated board presented a very pleasing appearance.

SOME OF THE PHYSICAL-CHEMICAL PROPERTIES OF HOG CHOLERA VIRUS[1]

I. FILTERABILITY OF HOG CHOLERA AS AFFECTED BY THE HYDROGEN ION CONCENTRATION

II. THE MIGRATION OF HOG CHOLERA VIRUS WHEN SUBJECTED TO ELECTROPHORESIS

III. EXPERIMENTS ON THE ATTENUATION OF VIRUS AND THE PRODUCTION IMMUNITY TO HOG CHOLERA

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PART I. FILTERABILITY OF HOG CHOLERA VIRUS AS AFFECTED BY THE HYDROGEN ION CONCENTRATION

The filters selected for these studies included the standard laboratory filters such as the regular Mandler, Berkfeld No. 3 "N", Chamberland-Pasteur "F" and the Seitz. Gypsum filters made according to Kramer's formula and compound filters made of a gypsum cortex built around Berkfeld No. 3 "W" filter candles were also used in these studies. The pH determinations were made with the glass electrode. The experimental results are summarized in the following tables.

CONCLUSIONS

1. Serum virus of hog cholera passes readily through the regular Mandler, Berkfeld "N", Chamberland-Pasteur "F" and Seitz filters, which are all standard. It also passes readily through the gypsum and compound filters which were made in the laboratory.

2. These filters were efficient in removing bacteria from liquid suspensions.

3. The volume of the filtrate delivered by individual filters of the same type under identical pressures varied considerably.

4. The studies on the effect of pH upon the filterability of hog cholera serum virus showed that within the range used, pH = 5.0 to 9.0; this factor is not of practical significance.

PART II. THE MIGRATION OF HOG CHOLERA VIRUS WHEN SUBJECTED TO ELECTROPHORESIS

The apparatus used was similar to that used by Todd, with some modifications. The two side arms of a large three-way stopcock were turned to a vertical position two inches from the center tap. Pyrex test tubes, 2 x 15 cm., were drawn out from the base and welded to the three arms of the stopcock in a vertical position. The side chambers of the apparatus were connected by two inverted U tubes, with two small glass

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TABLE 1. *Experiments with siliceous filters*

Filter	Serum virus	pH	Volume delivered	Temperature	Test pig	Symptoms	Diagnosis
Mandler (regular)	3903	5.0	45 cc.	22° C.	3928	6th day	H. C.
Mandler (regular)	3903	6.0	40.2 cc.	22° C.	3929	5th day	H. C.
Mandler (regular)	3903	7.0	47.0 cc.	22° C.	3930	5th day	H. C.
Mandler (regular)	3903	8.0	52.2 cc.	22° C.	3931	5th day	H. C.
Mandler (regular)	3931	9.0	45.0 cc.	24° C.	3901	4th day	H. C.
Berkfeld "N"	3931	5.0	67.5 cc.	24° C.	3904	6th day	H. C.
Berkfeld "N"	3930	6.0	124.8 cc.	21° C.	3934	4th day	H. C.
Berkfeld "N"	3934	7.0	102.2 cc.	24° C.	3949	4th day	H. C.
Berkfeld "N"	3942	8.0	86.4 cc.	29° C.	3905	5th day	H. C.
Berkfeld "N"	3942	9.0	101.6 cc.	29° C.	3951	4th day	H. C.
Chamberland-Pasteur "F"	3942	5.0	43.5 cc.	31° C.	3950	5th day	H. C.
Chamberland-Pasteur "F"	3909	6.0	51.2 cc.	23° C.	3957	4th day	H. C.
Chamberland-Pasteur "F"	3909	7.0	47.6 cc.	24° C.	3958	6th day	H. C.
Chamberland-Pasteur "F"	3971	8.0	42.0 cc.	23° C.	3972	8th day	H. C.
Chamberland-Pasteur "F"	3971	9.0	54.1 cc.	23° C.	3979	8th day	H. C.

Vacuum maintained at 350 mm. Hg.
Time—3 minutes.
H. C. = positive diagnosis of hog cholera.

TABLE 2. *Experiments with gypsum filters*

Filter	Serum virus	pH	Volume delivered	Temperature	Test pig	Symptoms	Diagnosis
Gypsum	3971	5.0	14.1 cc.	23° C.	3978	9th day	H. C.
Gypsum	3979	6.0	16.5 cc.	22° C.	3967	5th day	H. C.
Gypsum	3971	7.0	12.2 cc.	23° C.	3976	7th day	H. C.
Gypsum	3971	8.0	18.2 cc.	22° C.	3977	12th day	H. C.
Gypsum	3977	9.0	15.4 cc.	20° C.	3996	5th day	H. C.

Vacuum maintained at 350 mm. Hg.
Time—20 minutes.
H. C. = Positive diagnosis of hog cholera.

TABLE 3. *Experiments with compound filters*

Filter	Serum virus	pH	Volume delivered	Temperature	Test pig	Symptoms	Diagnosis
Compound	3996	5.0	36.5 cc.	21° C.	3998	5th day	H. C.
Compound	3996	6.0	41.3 cc.	21° C.	3999	5th day	H. C.
Compound	3999	7.0	34.8 cc.	22° C.	4000	6th day	H. C.
Compound	4708	8.0	44.2 cc.	20° C.	4003	10th day	H. C.
Compound	4708	9.0	40.1 cc.	22° C.	4010	4th day	H. C.

Vacuum maintained at 350 mm. Hg.
Time—20 minutes.
H. C. = Positive diagnosis of hog cholera.

TABLE 4. *Experiments with Seitz–Uhlenhuth type of filters*

Filter	Serum virus	pH	Volume delivered	Temperature	Test pig	Symptoms	Diagnosis
Seitz–Uhlenhuth	4010	5.0	14.0 cc.	21° C.	4711	7th day	H. C.
Seitz–Uhlenhuth	4714	6.0	16.4 cc.	22° C.	4715	6th day	H. C.
Seitz–Uhlenhuth	4714	7.0	18.6 cc.	20° C.	4716	5th day	H. C.
Seitz–Uhlenhuth	4716	8.0	15.2 cc.	21° C.	4726	6th day	H. C.
Seitz–Uhlenhuth	4716	9.0	16.5 cc.	21° C.	4727	6th day	H. C.

Vacuum maintained at 350 mm. Hg.
Time—5 minutes.
H. C. = Positive diagnosis of hog cholera.

bottles containing non-polarizable electrodes. These tubes were filled with 1 per cent saline in 2 per cent agar. The cathode made of copper wire was submerged in dilute copper sulphate solution. The anode constructed of iron wire was submerged in a solution of ferric sulphate. The apparatus was sterilized after the cotton plugs had been placed in the lower side arm tubes. The sterile buffer solutions adjusted to the desired pH were placed in the side chambers of the apparatus. The serum virus after being adjusted to the proper pH was placed in the center chamber. The direct current was supplied by a motor generator and the current was measured by a milliammeter. The pH values were determined by a glass electrode. The experimental results are summarized as follows:

Experiment	pH	Positive pole	Center chamber	Negative pole
I	5.0	+	+	—
II	6.0	+	*—	—
III	7.0	+	+	—
IV	8.0	+	+	—
V	9.0	+	+	—

+ Indicates the presence of active hog cholera virus as determined by inoculation into susceptible pigs.

— Indicates the absence of active hog cholera virus.

* Experimental pig immune to hog cholera.

Milliamps—20.

Time—3 hours.

Temperature—37° C.

The protein tests showed the presence of serum proteins in the liquid taken from the center chamber of the apparatus and also in that which was taken from the positive pole.

CONCLUSIONS

1. The serum virus of hog cholera migrates toward the positive pole at pH values from 5.0 to 9.0.

2. The virus of hog cholera either carries a negative electric charge or is carried toward the positive pole by the associated proteins.

3. It is not possible within the pH range studied to separate hog cholera virus from the associated proteins by the electrophoretic method employed.

PART III. EXPERIMENTS ON THE ATTENUATION OF VIRUS AND THE PRODUCTION OF IMMUNITY TO HOG CHOLERA

DESICCATED TISSUES

Brain and spinal cord tissues were taken from a cholera infected pig and desiccated. When this preparation no longer proved virulent a sus-

ceptible pig was injected with two 10 cc. units of a 50 per cent suspension in a diluting agent consisting of equal parts of glycerine and physiologic saline solution. These injections were made at ten-day intervals. The test animal later proved to be susceptible to hog cholera.

Desiccated serum virus was tested until it was no longer infectious. A susceptible pig was likewise injected with two 10 cc. units of a 50 per cent suspension in physiologic saline solution. The test animal was later proven to be susceptible to hog cholera.

FORMALIZED TISSUES

Liver, spleen and brain tissues taken from cholera infected swine were finely triturated and inactivated with 1 per cent formalin. Susceptible pigs were injected with two 10 cc. units of a 50 per cent tissue suspension in a diluting agent consisting of equal parts of glycerine and physiologic saline solution. These injections were made at ten-day intervals. The test animal proved to be susceptible to hog cholera.

PHENOLIZED TISSUE

Tissues taken from the liver, spleen and brain of cholera infected swine were finely triturated and inactivated with 2 per cent phenol. Suscptible pigs were injected at ten day intervals with two 10 cc. units of a 50 per cent tissue suspension in a diluting agent composed of equal parts of glycerine and physiologic saline solution. These animals subsequently proved to be susceptible to hog cholera.

SATURATION OF VIRUS WITH GASES

Blood from cholera infected swine was diluted with equal parts of sterile distilled water and then saturated with gas. The gases used were nitrogen, chlorine, sulphur dioxide, oxygen, carbon dioxide and hydrogen. In the virulence test the pig injected with the carbon dioxide preparation contracted hog cholera. The hydrogen and oxygen dioxide preparations caused a slight temperature reaction in the susceptible pigs, but produced no symptoms. These pigs were injected with two 10 cc. units of these preparations at ten-day intervals. The immunity test, which was conducted ten days following the last injection, resulted in cholera infection in all pigs except those injected with the oxygen and hydrogen preparations.

CONCLUSIONS

1. The preparations made from infective tissues inactivated by desiccation apparently have no value in producing immunity against hog cholera.

2. The preparations made from fresh tissue inactivated by phenol and formalin in the concentrations used did not seem to be effective in producing any degree of immunity.

3. The saturation of blood of infected swine with certain gases inactivates the virus.

4. The preparation attenuated or inactivated by oxygen and hydrogen apparently have some value in producing immunity against hog cholera.

THE BIOCHEMISTRY OF SLUGGISH BUTYL-ACETONIC FERMENTATIONS[1]

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In a normal butyl-acetonic fermentatoin there are three stages: 1, there is a vigorous bacterial reproduction and acid formation; 2, the acidity decreases with the parallel production of the solvents n-butanol, acetone and ethanol, in very nearly the ratio 6:3:1; 3, the acidity rises slowly, approaching a practically constant level. Two principal types of abnormal fermentations are of special importance in the industrial process, those caused by bacterial contamination, and the sluggish fermentations characterized by a prolonged acidity peak, decreased yields of solvents, incomplete utilization of fermentable carbohydrate and a generally slow fermentation. If a normal fermentation is inoculated with a small amount of material from a sluggish culture the normal culture may become sluggish. If a sluggish culture is filtered through a bacterial filter, a very small amount of filtrate may cause a normal culture to become sluggish; this type of sluggishness can be transmitted serially by means of the use of the filtrate. The sluggish principle has some of the characteristics of bacteriophage, but experiments have so far shown no evidence of lysis. A large commercial industry suffered heavily in 1923 through what was termed as "epidemic sluggishnes." Legg[2] patented a process for 'immunizing" the cultures against sluggishness.

The purpose of this thesis was three-fold:

1. To study the properties of the sluggish principle; 2. to study the biochemistry of the sluggish fermentaion; 3. to study various methods for the elimination of the sluggishness.

EXPERIMENTAL

Fifteen cultures were studied as to their susceptibility to sluggishness. There was a wide variation in resistance from extremely susceptible to wholly resistant.

The toxic filtrate, containing the sluggish principle, was prepared from a culture freshly isolated from wheat. It may be stated that many trials are necessary to find a sluggish culture, indicating that the sluggish principle is not produced by the bacteria, but is occasionally associated with it in the material used for isolation. A very satisfactory filter was the Chamberlain-Pasteur filter chamber designated as L5.

Studies on the effect of pH on filterability showed values of 4.5-9.0 suitable, but the optimum range for storage was pH 5.6-6.0; filtrates more acid than pH 5.3 were badly deteriorated in potency after three months storage at room temperature.

Two strains of the bacteria were subjected to Legg's immunization procedure, but after 17 treatments did not develop resistance. Eight cultures were subjected to a modification of the above method. One of the cultures developed a high resistance.

Studies on the effect of the filtrate on the course of the fermentation of 5 per cent corn mash showed that in the presence of the sluggish principle the bacterial counts are lower during the first part of the fermentation, but may exceed the normal counts at the end of the fermentation; the reduction of methylene blue is slower in the initial stages but more rapid in the final stages of the fermentation than normally; the production of reducing sugar is slower and does not attain as high a value as in the normal fermentation. There is no difference in the action on gelatin as measured by viscosity and formol titration. The maximum gas production occurred 15 to 20 hours later than in the normal fermentation. There is sufficient difference between the thermal death points for the bacteria and the sluggish principle to suggest that one advantage in heat shocking spores before starting a fermentation may be due to the destruction of the sluggish principle.

Dilution experiments with the toxic filtrates showed that 1,000 units per cc. could be definitely demonstrated and that over 100,000 units of the sluggish principle were present in some filtrates.

THE BUTYL-ACETONIC FERMENTATION OF THE SUGARS WITH SPECIAL REFERENCE TO XYLOSE[1]

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The commercial development of the butyl alcohol fermentation industry marks a big step in the utilization of agricultural products in the manufacture of industrial chemicals. This industry, just before the depression, utilized about 30,000,000 bushels of corn annually, the principal use of the butyl alcohol being in the preparation of nitrocellulose lacquers. The finish on the average automobile represents the fermentation products of one and three-quarters bushels of corn. The starch is transformed into the solvents, butanol, acetone, and ethanol in the approximate ratio of 6:3:1, together with carbon dioxide and hydrogen. The latter two products are used in the synthesis of methanol. The principal raw material is corn.

Quantitative studies on the butyl fermentation of carbohydrates other than starch, are limited. The material presented in this thesis deals with the butyl fermentation of several carbohydrates, especially xylose. This latter sugar, produced by the hydrolysis of pentosan-containing materials, is probably the cheapest carbohydrate for this process. It is obtained especially from agricultural wastes such as corn cobs, oat hulls, peanut shells, cottonseed hull bran, and straws. Due to methods developed by the United States Bureau of Standards, crystalline xylose is now economically available for quantitative studies on fermentation.

METHODS

The butyl organism was isolated from wheat and handled and stored by the usual technique. In practically all of the studies the fourth or fifth transfer from the original "soil-culture" was used for inoculation of the various media employed. The stock medium for carrying the culture was a 6 per cent corn mash. The following analyses were made at various periods of time during the fermentation: total acidity, total solvents, butanol, acetone, ethanol, volatile acids, carbon dioxide, hydrogen, and sugar unfermented. The yields were expressed in terms of the percentage of total glucose equivalent, that is, on the basis of the quantity of carbohydrate required to furnish the same amount of carbon as the glucose.

EXPERIMENTAL RESULTS

A. ACTION OF THE BUTYL ORGANISMS ON VARIOUS CARBOHYDRATES

1. *Replacement of corn meal by carbohydrates and sources of nitrogen*

Equivalent amounts of corn meal were replaced by starch, glucose, sucrose, and xylose. The meal could be replaced by the following per-

[1]Original thesis submitted June, 1934.

centage of the carbohydrates before decreased solvent yield was apparent: starch, 90 per cent; glucose or sucrose, 80 per cent; and xylose, 50 per cent.

Various sources of nitrogen were used in place of the corn meal, employing xylose as the substrate. These included peptone, tankage, steep water, corn-gluten meal, casein, and ammonium chloride. The ammonium chloride and steep water gave the poorest results; the best yields were obtained with the peptone and corn-gluten meal. The highest yields with the gluten meal were obtained in the medium containing 0.5 gram of the material per 100 cc. The addition of varying amounts of K_2HPO_4, $MnSO_4$, NaCl, $MgSO_4$ or $FeSO_4$ did not increase the yields.

2. The course of the butyl-acetonic fermentation of various carbohydrates

The medium contained 0.5 per cent corn-gluten meal together with the carbohydrates tested. These included starch, glucose, maltose, levulose, sucrose and xylose. At various periods of time determinations were made of the total acidity, pH, butanol, acetone, ethanol, total solvents, and sugar consumed. All of the sugars were attacked by the butyl organisms and at maximum solvent yields the ratio of solvents produced was that normal for corn mash, that is, about 6:3:1.. In general, the fermentations of the carbohydrates in the semi-synthetic medium were marked by greater time required, less pronounced acidity breaks, and somewhat poorer utilization of carbohydrates. The maximum total solvent yield showed little variation from that of corn mash.

A careful fractional analysis of the distillate showed that butanol, acetone, and ethanol are the only neutral volatile products formed in appreciable amounts.

B. INFLUENCE OF VARIOUS FACTORS UPON SOLVENT YIELD

1. Influence of surface-volume ratio

It has often been observed in practice that larger yields may be obtained in a large-scale fermentation than on a laboratory scale, a fact which might reasonably be associated with surface-volume ratio. The results of experiments to test this theory are given in table 1, from which it is evident that the solvent production does increase with decrease in surface-volume ratio.

TABLE 1. *Variation of yields of total solvents with change in the surface-volume ratio of the fermentations*

Flask size (cc.)	Vol. of medium (cc.)	Surface (sq. cm.)	Surface-volume ratio*	Yield, percentage of glucose equiv.		
				Corn	Glucose	Xylose
150	100	22.5	0.225	20.58	20.40	19.58
500	300	50	0.167	24.66	24.42	22.50
1,000	750	62	0.083	27.10	28.79	24.52
2,000	1,500	80	0.053	28.66	30.02	25.90
4,000	3,000	110	0.037	30.24	31.40	27.00

2. Prolonged incubation

It has been found in practice that after active fermentation has stopped the solvents decrease in amount, with accompanying rise in acidity, hence the solvents are distilled before this change takes place. It was found that the same phenomenon occurs in the fermentation of xylose in the semi-synthetic medium, as shown by the data in table 2.

TABLE 2. *Solvent yields from xylose*

Time in days	Acidity[1]	Solvent yield[2]
0	1.50	....
1	4.37	6.41
2	2.57	16.41
3	2.35	25.00
4	2.35	27.41
5	2.45	27.08
6	2.87	25.90
7	3.10	25.30
8	2.97	24.76
9	3.35	24.08
10	3.35	23.38
11	3.40	23.38
12	3.35	22.29
13	3.40	21.48

[1]Cc. of 0.1 N NaOH required for 10 cc. of medium.
[2]Yield of total solvents, percentage of glucose equivalent.

3. Inoculation from different transfers

It is common practice to use the fourth or fifth transfer to inoculate the mash for the production of solvents. Experiments were conducted to determine the influence of the number of transfers upon the solvent yield from xylose. The best yields were obtained by using from the second to the seventh transfer. The yields were materially less on the eighth and ninth transfers.

4. The continuous carbon balance

The course of the butyl-acetonic fermentation of xylose was determined with reference to the following items: total acidity, pH, xylose fermented, butanol, acetone, ethanol, carbon dioxide, butyric acid, acetic acid, and non-volatile acid. Data are given in table 3 for the carbon balance at various periods of time.

TABLE 3. *The carbon balance*

Time (hours)	Carbon in xylose (gram-atoms)	Carbon in products (gram atoms)
44	5.0	6.21
60	5.0	5.74
68	5.0	5.50
80	5.0	4.88
92	5.0	4.82
122	5.0	4.88
144	5.0	5.06

It is evident that the carbon recovered in the products during the early stages of the fermentation was considerably greater than the sugar consumed. This discrepancy may be associated with intermediates which also reduced the copper solution in the sugar analysis. After the fermentation had attained normal maximum yields the carbon balance approached the theoretical very closely.

THE PRODUCTION OF ALCOHOLS BY THERMOPHILIC FERMENTATIONS[1]

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Cellulose and cellulosic materials constitute a large proportion of such products as corn cobs, oat hulls, beet pulp, peanut hulls and the straws. One method for the utilization of these materials is their conversion into industrial chemicals by means of fermentation. This may be accomplished by direct bacterial action upon the cellulosic materials, or by the action of the appropriate microorganisms upon the hydrolyzed material. The most promising type of direct fermentation is that brought about at high temperatures, that is, the thermophilic fermentation. Two commercial developments have taken place in this field; the first, the production of combustible gases, and the second, the production of acetic acid.

A great deal of work has been done in the thermophilic fermentation of cellulose; most of the research has been directed toward the production of high yields of acetic acid and the isolation of pure cultures of the bacteria involved. Yields of acetic acid as high as 50 per cent of the cellulose added have been reported. A survey of the literature shows an occasional report of the production of ethyl alcohol by the thermophilic fermentation of cellulose, but little systematic work has been done with particular reference to the maximum production of this chemical. The purpose of this thesis was to determine the optimum conditions for the production of maximum yields of ethyl alcohol by the thermophilic fermentation of cellulose.

The cultures were obtained from several sources and were selected on the basis of rate of growth and ability to produce alcohols and volatile acids. It was noticed that some cultures gave relatively high alcohol yields while others gave relatively high yields of volatile acids. The cultures which produced the highest yields of alcohol came from a compost pile and a mixture of rotting horse manure and straw.

The ethanol and butanol were separated from the fermented liquor by neutral distillation, and were estimated by a wet oxidation method, using potassium dichromate. The volatile salts were separated by the distillation of an acidified sample, estimated by titration with standard hydroxide, and calculated as acetic acid. The ratios of acetic and butyric acid were determined by the method of Fyleman. Non-volatile acids were separated by the extraction with ether of the residual liquor from the volatile acid distillation, estimated by titration with sodium hydroxide, and calculated as lactic acid. Reducing sugars were determined by the method of Shaffer and Hartmann. The gaseous products were analyzed with a Williams gas analysis apparatus. The amount of residual cellulose was determined by treating a sample of the fermented liquor

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with hydrochloric acid, filtering, treating the residue with a hot sodium hydroxide solution, washing, drying and weighing.

Using media consisting of filter paper, ammonium chloride, di-potassium phosphate, excess calcium carbonate, and tap water, fermentations were conducted at temperatures ranging from 37.5° C. to 65° C. At 37.5° C. no growth was evident even after 50 days. The highest yield of alcohols was obtained at 55° C., and the maximum yield was reached in 8 days. The highest yield of acetic acid, on the other hand, was obtained at 60° C., and the maximum yield was reached in 11 days.

Systematic studies were made of the effect of the composition of the media upon the production of ethyl alcohol. The cultures had been grown for some time on media consisting of filter paper, ammonium chloride, di-potassium phosphate, and excess calcium carbonate in tap water, but it was not known whether the optimum concentrations were being used. When the concentration of ammonium chloride was varied, the largest yields of alcohols were obtained within the range of 0.20 to 0.55 g. of ammonium chloride (NH_4Cl) per 100 cc. When the concentration of di-potassium phosphate was varied, the yields of alcohols were greatest within the range of 0.20 to 0.40 g. of di-potassium phosphate ($K_2HPO_4 . 3H_2O$) per 100 cc. With variation in concentration of cellulose, little change in the efficiency of the conversion of cellulose to alcohols was noticed within the range of 2 to 5 g. of cellulose per 100 cc. At the conclusion of these experiments, a medium consisting of 3 g. of cellulose, 0.25 g. of ammonium chloride, and 0.25 g. of di-potassium phosphate per 100 cc. of tap water was adopted.

In most of the experiments calcium carbonate had been used as the neutralizing agent to react with the acids formed and regulate the pH; the use of calcium carbonate gave a pH value of 6.5 to 6.8. In order to determine the effect of pH, fermentations were adjusted twice daily with sodium hydroxide or sodium carbonate to pH values ranging from 5 to 9. The highest yields of alcohols were obtained when the adjustments were made to pH values of 7.5 to 8.0. Thus it was shown that the calcium carbonate had not been giving a reaction sufficiently alkaline to give maximum yields of the alcohols.

In further attempts to increase the yields of alcohols, it was found that aeration decreased the yield, and that the addition of 0.50 g. of peptone per 100 cc. increased the yield 28 per cent. The addition of glucose up to 0.25 g. per 100 cc. increased the yield of alcohols slightly, while larger amounts of glucose were inhibitory to the fermentation of cellulose. The presence of glucose did not increase the rate of decomposition of the cellulose. Continued growth on a glucose medium containing no cellulose caused the culture to lose its cellulose-fermenting power.

The alcohols produced during the fermentation were shown to be ethanol and n-butanol, in the ratio of about 20 to 1. The volatile acids consisted of acetic acid and butyric acid in the ratio of about 20 to 1. The gases were found to consist mainly of carbon dioxide and hydrogen. Sometimes very small amounts of methane were also formed. The percentage of hydrogen in the gases was greatest during the early stages of the fermentation. By using the best alcohol producing cultures in the synthetic medium described above, yields of 16 to 18 per cent, based on the cellulose added, were obtained quite regularly at 55° C. By adjusting

medium to a pH value of 7.5, the yields were increased to values of 20 to 25 per cent. A fermentation at 60° C., under otherwise optimum conditions, gave 26.6 per cent carbon dioxide; 24.4 per cent volatile acids, calculated as acetic acid; 26.3 per cent ethanol; 0.715 per cent butanol; 0.71 per cent reducing sugar; 0.356 per cent hydrogen; 0.244 per cent non-volatile acids, calculated as lactic acid; 0.027 per cent methane; 12.85 per cent of a material soluble in acids and insoluble in bases; and 3.22 per cent of unfermented cellulose. The total recovery was thus 95.422 per cent by weight.

THE ALKALINE OXIDATION OF LIGNIN[1]

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From the Department of Chemistry, Iowa State College

The purpose of the thesis centers around the study of the method of preparation of lignin from cornstalks and oat hulls by the use of aqueous ammonia, and the study of the oxidation of lignin with an alkaline iodine solution.

A short historical review on the general subject of lignin, together with several definitions of lignin, are given. The indefiniteness of the term is indicated.

The more general methods for the preparation of lignin are reviewed, including the acid, sulfite, alkali and neutral solvent methods. The non-uniformity of the lignin preparations are compared with one another, and the question of purity is emphasized.

The more important formulae presented in the literature for the possible structure of lignin are given. These formulae include those of Schrauth, Freudenberg, Dorée and Hall, Cross and Bevan, Fuchs, and Klason.

A short review of the literature bearing directly on the experimental procedures is given. The various oxidation products of lignin obtained by different methods of preparing and oxidizing alkali lignins, and those obtained by different investigators, are listed. The importance of the results is discussed.

Ammonia lignin was prepared from cornstalks by the following procedure: The tissues were cooked at 100 pounds pressure for eight hours with concentrated aqueous ammonia. The lignin and pentosans were precipitated with acid. The pentosans were removed by dilute acid hydrolysis. Yields, properties and the objections to the method were discussed.

A new method for preparation of alkali lignin from oat hulls was investigated. The method was carried out essentially the same as that for cornstalks, except that the hydrolysis of the pentosans preceded the alkaline extraction with ammonia.

The oat hull lignin was oxidized with alkaline hypohalite. Use of an alkaline solution of chlorine was found to give indefinite products. The same result occurred with alkaline bromine solution, but crystalline carbon tetrabromide was characterized and identified as one of the oxidation products.

Alkaline solutions of iodine were found to react quantitatively with lignin. The effect of time on the iodine equivalent was studied; oxidation was complete and remained constant after thirty-six minutes. Iodoform was proven to be one of the oxidation products.

Lignin oxidized by alkaline iodine was prepared and studied. The methoxyl content was found to be 7.2 to 7.4 per cent and the ash content

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[1]Original thesis submitted December, 1933.

0.5 per cent. A comparative table of the properties of the oxidized and the unoxidized lignin is given. Methylated lignin was found not to oxidize in an alkaline iodine solution. Nitrolignin could not be oxidized quantitatively.

Acid hydrolysis did not appreciably affect the iodine equivalent of oat hull lignin. Sugars could not be identified in the solution.

Oat hull lignin was oxidized by Fehling's solution. Analysis of the copper reduced corresponded to a dextrose equivalent of 5.4 per cent. The change in the iodine equivalent was 34 cc. of 0.1 N iodine per gram sample.

Sulfuric acid lignins from spruce wood, aspen wood and oat hulls were found to have iodine equivalents of 68, 112 and 160 cc. of 0.1 N iodine solution per gram sample, respectively. Iodoform was present in the oxidized solution of each.

Oxidized oat hull lignin containing 7.23 per cent of methoxyl was methylated with dimethyl sulfate. The product contained 22.19 per cent of methoxyl and was found to be insoluble in cold sodium hydroxide solution. When the methylated product, suspended in 10 per cent sodium hydroxide solution, was warmed to approximately 80° C. it began to go into solution, and at 90° C. was completely soluble in the alkali. The alkali-soluble product was reprecipitated with dilute sulfuric acid and was found to contain 17.4 per cent of methoxyl and 10.5 per cent iodine.

Five different tissues were oxidized with an alkaline iodine solution both before and after hydrolysis. The samples were dried at 105° C. for two hours. The acid hydrolysis was made with 0.1 N. hydrochloric acid at ten pounds pressure for six hours. The time of oxidation was increased to four hours and the end point in the titration was considered to be the point at which the starch solution remained colorless for five minutes. The lignin was determined at 4° C. by the 72 per cent sulfuric acid method; the methoxy content was determined by the Zeisel method. The oxidation values were based on the dry weight of the matrial analyzed. The results are given in the following table.

Calculation of the minimum molecular weight from the iodine and the methoxy contents gave 1,210 and 600, respectively, showing that there are two carboxyl groups present in oxidized oat hull lignin for every atom of iodine.

Some of the lignin and some of the methoxy groups disappeared on acid hydrolysis. Assuming that the methoxy groups and the iodine equivalent were due entirely to the lignin present, calculations were made on the approximate methoxyl content of the lignins.

The significance of the alkaline oxidation of lignin is emphasized in the discussion of the results. The formation of iodoform during oxidation indicates the presence of a methyl carbinol or a methyl ketonic group in lignin.

It is emphasized that the same methoxy contents were obtained from lignin prepared from different ammonia cooks. The methoxy contents of oxidized lignin were the same from the cold alkali extraction of acid hydrolyzed hulls as that from the ammonia cooking process. The iodine equivalent of the isolated lignin was of the same order of magnitude as that calculated for the lignin present.

TABLE 1. *The effect of acid hydrolysis on the alkaline-iodine oxidation equivalent, lignin content and methoxy content of several plant tissues*

	Oat hull percentage	Rye straw percentage	Flax straw percentage	Artichoke stalks percentage	Cornstalks percentage
ORIGINAL TISSUE					
Lignin	19.07	21.04	22.87	23.99	24.74
Methoxy	2.62	3.51	3.47	4.51	3.11
Calc. methoxy in lignin	13.74	16.68	15.17	18.80	12.57
N/10 iodine equivalent, cc./gram sample	16.56	27.58	17.90	18.42	39.90
N/10 iodine equivalent, cc./gram lignin	86.80	131.10	78.30	76.80	161.30
HYDROLYZED TISSUE					
a. Unhydrolyzed tissue	52.54	57.38	60.85	56.22	50.90
Lignin	27.46	33.86	34.53	37.23	43.79
Methoxy	3.77	4.92	4.92	6.01	4.34
Calc. methoxy in lignin	13.73	14.53	14.25	16.14	9.91
N/10 iodine, cc./gram sample....	47.71	57.11	35.52	51.85	71.50
N/10 iodine, cc./gram lignin......	171.20	168.60	103.90	139.20	163.30
b. Calc. on basis of original tissue—					
Lignin	14.43	19.43	21.01	20.93	22.29
Methoxy	1.98	2.82	2.99	3.38	2.21
LOSS DUE TO HYDROLOSIS					
Lignin	4.64	1.61	1.86	3.06	2.45
Methoxy	0.64	0.69	0.48	1.13	0.90
Methoxy in lignin	0.01	2.15	0.92	2.66	2.66
FRACTION OF TOTAL HYDROLYZED					
Lignin	24.33	7.65	8.13	12.75	9.90
Methoxy	24.40	19.60	13.80	25.10	28.90

STUDIES ON THE ESCHERICHIA-AEROBACTER GROUP OF BACTERIA IN DAIRY PRODUCTS[1]

Maurice Wade Yale

From the Department of Dairy Industry, Iowa State College

The Escherichia-Aerobacter group of bacteria was studied on a species basis with respect to numbers in dairy products, development of off-flavors in experimental butter and action on milk constituents.

Bergey's classification of the group was revised to agree with original descriptions and *Escherichia foetida* (Perez), *E. noctuarii* (White), *E. sphingidis* (White), *E. ichthyosmia* (Hammer), *E. iliaca* (Ford) and *Aerobacter bombycis* (Glaser) were dropped from the group because original descriptions stated that they did not ferment lactose. *E. schaefferi* (von Freudenreich) was considered synonymous with *E. coli* (Escherich).

Two hundred and four cultures belonging to the Escherichia-Aerobacter group were isolated from 212 samples of dairy products as follows: 91 cultures from 70 samples of raw milk; 21 from 64 samples of pasteurized milk; 42 from 24 samples of raw cream; 16 from 20 samples of ice cream; 9 from 9 samples of ropy milk and cream; and 25 from 25 samples of defective butter.

The genus Escherichia comprised 63 per cent of the cultures from raw milk; 57 per cent from pasteurized milk; 33 per cent from raw cream and 31 per cent from ice cream.

The genus Aerobacter comprised 26 per cent of the cultures from raw milk; 10 per cent from pasteurized milk; 57 per cent from raw cream; 56 per cent from ice cream; 100 per cent from ropy milk and cream; and 88 per cent from defective butter.

Ten of 29 species included by Bergey in the genus Escherichia were identified, viz., *E. coli*, *E. pseudocoloides*, *E. communior*, *E. paragrünthali*, *E. vesiculiformans*, *E. formica*, *E. enterica*, *E. anaerogenes*, *E. grünthali* and *E. neapolitana*. Three of 6 species included by Bergey in the genus Aerobacter were identified, viz., *A. aerogenes*, *A. cloacae* and *A. oxytocum*. *E. coli* was the most common species in raw milk; *E. pseudocoloides* in pasteurized milk; *A. cloacae* in ice cream; and *A. aerogenes* in raw cream, ropy milk and cream, and defective butter. Of 80 cultures that could not be identified on the basis of species in Bergey's classification 26 belonged to an intermediate group.

While 0.01 cc. quantities of raw milk from composite supplies usually showed the presence of organisms of the Escherichia-Aerobacter group, 10 cc. quantities of pasteurized milk taken from the pasteurizer showed their presence in only 1 out of 15 pasteurizations run at the College Dairy and in only 5 out of 17 runs at 13 other Iowa plants. In the latter case, 3 positive samples were known to have been improperly pasteurized.

At the College Dairy, organisms of the Escherichia-Aerobacter group were present in a larger percentage of cases and in higher numbers in the first milk bottled than in milk bottled at a later stage. The presence of the highest numbers of these organisms in the first milk bottled indicated that contamination from equipment was gradually reduced by the flow of the pasteurized milk. Similar results were obtained at the other pasteurization plants studied.

Both young and old cultures of *E. paragrünthali* and *A. cloacae* that were originally isolated from pasteurized milk were destroyed by 20 minutes heating at 62° C. (143.6° F.). Old cultures were slightly more resistant than young cultures.

The number of Escherichia-Aerobacter organisms in 24 samples of cream for butter making ranged between 25 per cc. and 600,000 per cc. The counts exceeded 10,000 per cc. in over 60 per cent of the samples. In 20 samples of ice cream from 11 commercial plants the number of these organisms ranged between 3 per cc. and 2,500 per cc. and exceeded 100 per cc. in 30 per cent of the cases.

Of 25 cultures isolated from 17 samples of off-flavored butter, 15 were identified as *A. aerogenes*, 4 as *A. cloacae*, 3 as *A. oxytocum* and 3 as belonging to an intermediate group.

Flasks of pasteurized cream were inoculated with cultures of *E. coli*, *E. communior*, *E. formico*, *E. paragrünthali*, *A. aerogenes*, *A. oxytocum* and *A. cloacae*. Churnings of butter were divided into two lots, one of which was salted. Each lot was divided into two portions, one of which was held at about 7° C., and the other at about 18° C.

In 10 days at about 7° C., species of the genus Escherichia did not grow in salted butter, but in unsalted butter some of them did, while species of the genus Aerobacter sometimes grew in the salted butter and regularly grew in the unsalted.

In 10 days at about 18° C., species of both genera grew in salted as well as unsalted butter, but those of the genus Aerobacter grew more rapidly and reached higher numbers than did those of the genus Escherichia.

In 10 days at about 7° C., species of the genus Escherichia did not develop off-odors and flavors in butter, either when salted or unsalted; species of the genus Aerobacter did not develop off-odors and flavors in salted butter, but sometimes did in unsalted butter.

In 10 days at about 18° C., species of the genus Escherichia did not develop off-odors and flavors in butter either when salted or unsalted; species of the genus Aerobacter regularly developed off-odors and flavors in either salted or unsalted butter.

The unclean odor and flavor produced in butter by species of the genus Aerobacter definitely resembled the condition produced in milk by these organisms.

E. coli, E. communior, E. formica, A. aerogenes and *A. oxytocum* did not attack butter fat or cause appreciable proteolysis.

E. coli, E. communior and *E. formica* produced volatile acidities ranging from 68.0 to 80.5 (number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1,200 gram portion of fermented milk), while *A. aerogenes* and *A. oxytocum* produced volatile acidities ranging from 25.9 to 48.0. With the addition of 0.2 and 0.4 per

cent citric acid the species belonging to the genus Escherichia produced increased volatile acidities, while the species belonging to the genus Aerobacter in most cases showed a slight decrease. The volatile acids produced by species of the genus Escherichia were largely acetic with small amounts of propionic as determined by the percentages of barium in the barium salts while they were entirely acetic as determined by Duclaux values.

SOME QUANTITATIVE STUDIES OF ORGANOMETALLIC COMPOUNDS[1]

ERWIN A. ZOELLNER

From the Department of Chemistry, Iowa State College

This thesis has for its purpose the consideration of: First, a survey of the available literature on the comparative utility of the RMgCl, RMgBr and RMgI compounds in the practical application of the Grignard reagents; second, a study of the preparation of a number of organomagnesium chlorides, bromides and iodides, under general or special conditions; and third, the preparation of methyl-lithium from methyl chloride and n-butyl-lithium from n-butyl iodide.

The survey of the available literature, on the comparative utility of the RMgCl, RMgBr and RMgI compounds shows, in general, that the best results are obtained with the RMgCl and RMgBr compounds, and of these two, the RMgCl compounds are somewhat superior.

Methyl- and ethylmagnesium chlorides have been prepared from the gaseous halides in a closed system and under a pressure of 50 mm. Otherwise, the apparatus was that generally used for the preparation of Grignard reagents. The yields of the above Grignard reagents were 84.0 and 90.3 per cent, respectively.

Studies have been made of several alkylmagnesium chlorides in the same apparatus and under the same two conditions previously described[2]. In these studies, 0.05 mole of halide was reacted with 0.055 atom of commercial magnesium turnings. Under the first condition[2a], the halides were added to the magnesium in ether over a period of thirty to thirty-five minutes and under the second condition [2b], the halides were added all at once. The yields of the RMgCl compounds are given below with hydrocarbon radicals of the Grignard reagents (yields under the second condition are given in parenthesis): n-Propyl, 87.5; iso-propyl, 80.0; n-butyl, 91.9 (91.7); iso-butyl, 84.0 (77.2); sec-butyl, 90.1 (88.2); tert-butyl, 45.0 (40.0); α-sec-hexyl, 89.2 (83.2); β-sec-hexyl, 102.0 (96.7); β-phenylthyl, 88.9 (87.9); γ-phenylpropyl, 87.2 (85.3 per cent). When the dropping funnel and condenser were kept cold, the yield of iso-propylmagnesium chloride was 88.8 per cent.

A higher concentration of the alkyl chlorides in ether was necessary to start the reactions with magnesium in the usual manner, than that required for the corresponding alkyl bromides and iodides. Since the alkyl chlorides reacted slower than the corresponding alkyl bromides and iodides with magnesium, it took approximately an hour longer to carry out the reactions of the alkylmagnesium chlorides than the corresponding RMgBr and RMgI compounds.

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[2](a) Gilman, Zoellner and Dickey, *J. Am. Chem. Soc.*, 51:1576 (1929); (b) *ibid.*, 51:1583 (1929).

In view of the data given above on the survey of the comparative utility of the RMgCl, RMgBr and RMgI compounds and in consideration of the generally superior yields of the alkylmagnesium chlorides over the corresponding RMgBr and RMgI compounds, it is advisable to use the RMgCl compounds in the practical application of the Grignard reagents whenever these can be conveniently prepared.

Allylmagnesium chloride was prepared, under the same conditions used in this laboratory for the preparation of the corresponding RMgBr and in the same yield (90 per cent), by slow addition of the halide to excess of 30-80 mesh magnesium powder. More than 75 per cent of the runs were lost, however, due to the fact that the reaction mixtures became colloidal. Since this difficulty has, so far, not occurred in the preparation of allylmagnsium bromide, it is not now advised to replace the RMgBr by the RMgCl compound.

Studies have been made of the preparation of a number of organomagnesium bromides under the conditions of the alkylmagnesium chlorides given above. The yields of these organomagnesium bromides are also given in the same manner: Lauryl, 85.3 (74.3); cetyl, 72.6-79.5; β-phenylethyl, 89.3 (82.7); 2-cymyl, 87.0 (82.7); o-methoxyphenyl, 99.3 (96.3); p-methoxyphenyl, 91.2 (82.5); o-ethoxyphenyl, 98.8 (98.5); p-ethoxyphenyl, 84.5 (78.5); p-bromophenyl, 103.3 (99.8); p-chlorophenyl, 95.8 (91.2); β-styrly, 50 (42 per cent).

It was found that p-diphenyl- and mesitylylmagnesium bromide could be best prepared in 0.05 mole runs with commercial magnesium turnings, by adding all the halide to the magnesium at once. In this manner, the p-diphenylmagnesium bromide was prepared in a six-hour period of refluxing and stirring in yields of 75 to 77 per cent, and of mesitylylmagnesium bromide in yields of 82 to 83 per cent in a three hour period of refluxing. The yields were not improved by the use of fine magnesium. It was found difficult to start the reaction of mesitylylmagnesium bromide without the use of activated 12.75 per cent copper-magnesium alloy. With this activator the reaction started immediately and the yields were slightly improved.

Under comparable conditions, tert-butylmagnesium chloride, bromide and iodide were obtained in yields of 83.; 66.6 and 56.0 per cent, respectively, by slow addition of the halides to three equivalents of 30-80 mesh magnesium powder. Under the above conditions, the yield of β-styrylmagnesium bromide was 85 to 90 per cent. When three equivalents of ordinary magnesium turnings were used, the yield was 75 to 85 per cent.

Studies have been made of several organomagnesium iodides under the same conditions used for alkylmagnesium chlorides. The yields are also given in the same manner: Methyl, 90.3 (90.1); ethyl, 87.5 (78.5); n-propyl, 80.7 (71.4); n-butyl, 80.1 (62.0); n-amyl, 78.2 (58.2); n-nonyl, 66.2-70.1; n-decyl, 51.1-58.8; n-undecyl, 69.5-71.5; cetyl, 69-77; phenyl, 85.6 (72.3); 2-furyl, 87.9 (86.6); 2-methyl-5-furyl, 89.9 (91.3); 2-thienyl, 98.6 (99.7 per cent).

Methyl-lithium was prepared under the conditions and in the apparatus used for the preparation of methyl- and ethyl-magnesium chlorides, in a yield of 88.0 per cent, based on the halide used, and 89 per cent based on the lithium used. n-Butyl-lithium could not be prepared from n-butyl iodide.

Commemorating Six Decades of the Modern Era in Botanical Science

November 15 and 16, 1934

THE era is marked by the beginning of modern teaching and research in the botanical sciences. Following the brilliant contributions of de Bary, Darwin, Pasteur, Max Schultze, Sachs, and Asa Gray, during the fifties and sixties of the last century teaching received new impetus through the introduction into the laboratory of the compound microscope. Research was freed from the fetters of biased thinking. Modern concepts began to yield new knowledge and to give birth to new sciences.

CHARLES EDWIN BESSEY AND HIS MICROSCOPE

THE teaching of botany received strong impetus through the labors of Doctor Charles Edwin Bessey, who served Iowa State College from 1870 to 1884. Probably his greatest achievement in teaching was the stimulus he gave to the initiation of modern laboratory work when he introduced the use of the compound microscope.

This compound microscope, made by Beck and Son, England, was purchased for Doctor Bessey at a cost of $1,200.00. In 1882 when the building housing the botanical laboratories was partially demolished by a cyclone, the microscope was rescued from the wreckage by Vice President Herman Knapp, then a junior student. Doctor Bessey's first concern for his laboratory after the storm was this much cherished microscope. The instrument was highly prized for a long time as the most valuable piece of technical apparatus owned by the college. All students in his classes learned of this microscope, which Doctor Bessey used in his teaching and research while he served Iowa State College.
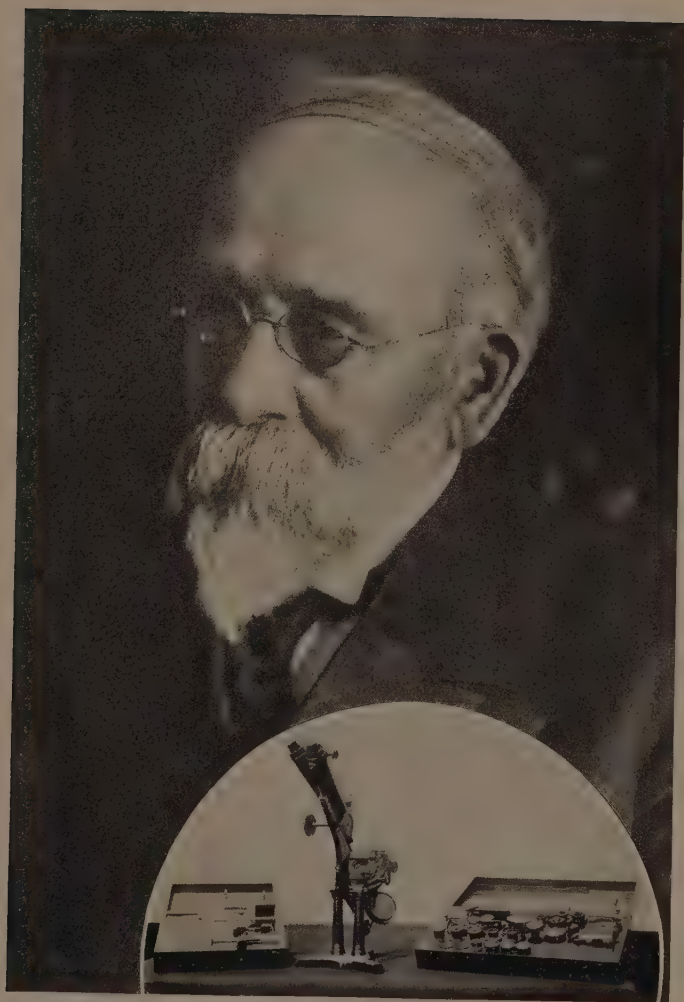
1. Symposium: Teaching General Botany

If it is agreed that the largest responsibility of botanists to their science and to society is in the field of teaching in its broadest sense, then it is fitting and timely that we gather around the conference table and weigh the place of the old in the light of the new. To this end this symposium has been arranged and it is hoped that through critical constructive thinking we may be able to reflect new light on some of the human relations of plants.

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COMMEMORATING SIX DECADES OF THE MODERN ERA IN BOTANICAL SCIENCE

I. E. MELHUS

From the Department of Botany, Iowa State College

We have chosen to call this occasion the commemoration of six decades of the modern era in botanical science. In initiating these exercises, we want you, our guests, to know that the Botany Department and the Corn Research Institute of the Iowa Agricultural Experiment Station feel honored by your presence. It is our profound wish that you may find your stay among us pleasant and profitable. This department is pausing in the midst of its work to commemorate the sterling services of a great teacher and to give thoughtful consideration to some of its major problems. Your presence and your contributions will prove most stimulating and helpful.

The beginning of the modern era takes us back six decades to the early seventies of the last century, when our science was beginning to respond to the influence of the epoch-making discoveries and labors of deBary, Darwin, Pasteur, Max Schultze, Sachs, Asa Gray, and others. It was at just this time that the botanical work in this institution was started.

A young man with a keen interest in plants came from an older school, Michigan Agricultural College, in 1870, to initiate and to carry on for a time the botanical teaching and research in this institution. The selection of Dr. Charles Edwin Bessey for this post was a most fortunate one. In him were combined the spirit of the pioneer, the traits of a great teacher and the rare ability to give definite direction and leadership in the botanical sciences.

In his new responsibility in a young institution, Dr. Bessey directed his attention to formulating his courses, and teaching the students that were committed to his charge. The courses that he formulated were in harmony with the precepts of a land-grant institution. This is well illustrated in the following courses of instruction in botany and horticulture for the junior year in 1871. The subjects required were: first, vegetable physiology; second, an examination of the more important useful plants; and, third, lectures on weeds and parasitic fungi. Regarding this last-named course, Dr. Bessey made this significant comment: "During the whole course the subject is illustrated by means of a good microscope, the College Herbarium, and a large collection of fungous plants."

In the fourth biennial report in 1873, this description of the first year of botany occurs. "During the first year of the course, students acquire a knowledge of the principles of structural botany from the study of 'Gray's Lessons,' as well as by actual dissection and analysis of plants." This is probably sufficient to indicate clearly that Dr. Bessey was mindful of the fundamental principles, but never neglected the applied aspects of his subject, and that he believed that the microscope and all other available means of illustrating his subject should be at the disposal of the student. During the 14 years that Dr. Bessey served this institution he set in motion forces that gave purpose and direction to the botanical work of this in-

stitution. In 1885 Dr. Bessey was called to the University of Nebraska, where he started anew. It is well known that it was in this institution where Dr. Bessey's life work flowered and bore abundant fruit, in the form of the Bessey school of botany, which has left a lasting interest in plants with thousands of students.

At this point I wish to raise with you the question as to whether we, as teachers, are utilizing our extensive resources of books, monographs, herbaria, museums, projection apparatus, charts and greenhouses to the same extent that Dr. Bessey did in the early seventies. As we look in retrospect, what changes and improvements have been effected? Since Bessey's time, many new plant sciences have taken their place in our college curricula. New subject matter, new points of view, advances in methods of teaching, large masses of students, and much too often a limited staff, are merely some of the problems which confront us today that did not exist six decades ago. How many of us are studying our teaching problems with the same vim and enthusiasm that we delve into problems of form, function, anatomy and taxonomy? It hardly needs to be said, that today we are not justified in utilizing the same methods by which we were taught one, two or three decades ago.

As we survey the field, the studies that have been made of our teaching problems by botanists themselves during the modern era are few and preliminary in scope. In fact, it seems that we have been content to permit the schools of education to do our experimental work, relating to teaching of our subject. In such cases, tests and trials have sometimes been made by teachers poorly trained in botany. We lament the situation, but still we do little about it. If botany is to hold its place in our curricula at the different levels, we must give critical study to our teaching problems. Only in this way can we fully succeed in leaving with the student at the close of our general courses a lasting interest in plants.

Not only did Dr. Bessey give direction to teaching in this institution, but also to research. His first efforts were to explore and to record the plant cover in the vicinity of this new institution in 1871. Although his interests were chiefly in the field of systematic botany, he contributed to the morphology and physiology of the flowering plants and fungi. Again, as in his teaching, so in research he was mindful of the economic aspects of his science, as is apparent from his contributions to the Erysiphaceae, and upon the injuriousness of porcupine-grass.

In 1882 he read a paper before the State Horticultural Society entitled, "Diseases of Plants," in which he showed the dearth of available information in English relating to diseases of plants in the United States and England. He listed at that time fifteen treatises on plant pathology in France and Germany. The only papers in English dealing with diseases of plants was a series of articles in the Gardener's Chronicle by Reverend Berkeley. This article by Dr. Bessey was among the first to outline the scope of the field of plant pathology in this country.

There were new fields to be developed in the beginning of the new era and it is no different today. One of the fields that has recently come into sharp relief is that of the rôle of plant cover in erosion prevention. The emergency measures for the control of erosion have set in motion forces that call for direction in a field where only a limited amount of pertinent knowledge is available. We know our flora only in part. We know a little

of its distribution and associations; and even less about the biology, growth response and development as influenced by existing disturbed conditions. It is hoped that our discussions may stimulate wider interest in this field, especially among botanists.

Through the very generous cooperation of the Director of the Iowa Agricultural Experiment Station, it has been possible to arrange another symposium, Applied Botanical Research of Maize, as the first program of the newly created Corn Research Institute.

These symposia, two on teaching and two on research topics, have been selected for our commemoration exercises. Since our largest responsibility as botanists to our science and to society rests in the field of satisfying the intellectual needs of the people and in adding to the store of human knowledge, it is fitting that occasionally we pause in our work and gather around the conference table to weigh the place of the old in the light of the new, and to project for the future. At this time the commemoration program is declared in session.

TEACHING GENERAL BOTANY

CHARLES E. FRILEY

From the Division of Industrial Science, Iowa State College

In higher education, as in education at other levels, the teacher is the essential life of the institution, and the quality of the teaching is the most important measure of the success of the college in its efforts to produce intelligent citizens.

Interest and concern in the problems of improving instruction in our colleges and universities are of quite recent origin. For many years the idea prevailed, and in some parts of the country is still current, that a college or university teacher needs only a thorough knowledge of the subject matter to be taught. But within the past decade it has been generally recognized that the study of teaching methods is more and more important in the equipment of the successful college instructor. It is also increasingly clear that there are other essential qualities: enthusiasm, sympathy, understanding of young people, ability to interpret one's subject (which is the highest form of teaching), and above all that intangible but vital asset called personality.

Perhaps in no other field are the problems of college teaching more difficult than in the natural sciences. The greater part of the instruction in science is carried on in the laboratories, which became a significant factor in American higher education not more than sixty years ago. New teaching techniques had to be developed for the laboratory, markedly different from the traditional methods of the lecture and recitation. Naturally there has been a great deal of experimentation, and much of trial and error, in seeking the most effective means of presenting the sciences to college students.

This Symposium on the Teaching of Botany is an effort to bring into focus the best of recent study, thought and practice in teaching this important sector in the field of the biological sciences. It is hoped that this may be the inspiration for future conferences of a similar character.

THE TEACHING OF BOTANY SIXTY-FIVE YEARS AGO

Ernst A. Bessey

I hope that no one in the audience will assume from the title of this paper that I was personally familiar with the method of teaching Botany two-thirds of a century ago. In spite of the grayness of the hairs that I still retain, I renounce the distinction of such a hoary old age. I ventured to give this paper because I have had the very good fortune to have been associated with two botanists whose training was received in that period, but whose teaching contributed largely to the modern methods. I refer to my father, Charles Edwin Bessey (1845-1915) and to my predecessor at Michigan State College, William James Beal (1833-1924). My father's first botanical training was obtained under Professor A. N. Prentiss, Professor of Botany and Horticulture at Michigan State Agricultural College. After coming to Iowa State Agricultural College in February, 1870, he spent parts of three winters, 1872, 1873 and 1875, studying under Dr. Gray and Dr. Goodale at Harvard. Dr. Beal was graduated at the University of Michigan in 1859 and taught, with intervals for study, at Union Springs, New York, until 1868. From 1862 to 1865 he studied at Harvard at various periods, receiving his Bachelor of Science there in 1865. He devoted what time he was permitted to the study of Chemistry, under Dr. Eliot, later President of Harvard, Zoology under Agassiz and especially Botany under Dr. Asa Gray.

We are accustomed, at present, to look back on the days of 1865 to 1875 as days in which laboratory work in botany was unknown in this country. Dr. Beal wrote that during his sojourn at Harvard between 1862 and 1865, the botanical department did not own a compound microscope, but borrowed one owned by Lowell Institute. Dr. Gray occasionally took Dr. Beal and two other students into his private laboratory. It is needless to say that this is far from the modern laboratory, but yet it contained the basic idea of learning to know a thing by personal observation, not from books alone.

It must be remembered that there were only two men in 1865 who earned their living in this country as professors of botany, Asa Gray at Harvard and D. C. Eaton, at Yale. Torrey at Columbia taught botany, to be sure, but eked out his meager salary by assay work. Such other teachers of botany as there were, such as Winchell at the University of Michigan, Prentiss at Michigan Agricultural College, etc., were either Professors of Natural History or combined Botany and Horticulture or other subjects.

From the late 40's to the 60's and 70's of last century the Government sent out numerous exploring and surveying expeditions to the West. With a foresight not always to be found connected with Government affairs, botanical collections were made on almost all these trips, often by men with some botanical training. This wealth of material was submitted to the few botanists for study and thus it is not to be wondered at that Systematic Botany demanded the major portion of their attention and

interest. This was, naturally, reflected in their teaching. Thus, the chief subject matter of botany was limited to such materials as would apprise a student of sufficient morphology and anatomy, with a little physiology added, to enable him to undertake collection and identification and other systematic work with plants.

The two text-books used in colleges in this country during the period under discussion were Gray's First Lessons in Botany and Gray's Botanical Text Book, both completed in 1857 and undergoing many revisions. In the final revision of this Text Book, a three or four volume work was contemplated, of which actually two volumes appeared, the first by Dr. Gray and the second, on anatomy and physiology, by Dr. Goodale. This last revision, however, appeared later than the time covered by my paper.

It is not strange, considering Dr. Gray's training and experience, that the emphasis was placed upon the seed plants. In the Lessons the material is treated almost exclusively from the standpoint of external morphology. Out of the thirty-four lessons and 236 pages of the 1866 editon, not quite six lessons, less than 33 pages in all, are devoted to anatomy and physiology. Two-thirds of a page is the whole reference to "Cryptogamous or Flowerless Plants." The Text Book was much more comprehensive and went into the anatomy and physiology of plants much more deeply. Furthermore, out of the 555 pages of the fifth (1868) edition a little over ten pages were devoted to Cryptogamous Plants, of which two pages are devoted to the "Order Fungi," one page to the "Order Lichenes" and two pages to "Order Algae." It is not strange, therefore, that it required the influence of the work of the German botanists to arouse in this country a general interest in the lower plants. It must not, however, be forgotten that even before this period there were amateurs, often not holding any academic position, who were very able students of fungi, lichens, algae, etc., but their influence had hardly penetrated into academic circles.

The manner in which these books were used in instruction varied greatly. Too often a certain number of pages would be assigned from the Lessons and the recitation would proceed by the question and answer method. This, according to my father's account, was the way that the book was used, even at Harvard in 1872. The more nearly that the student's answer corresponded word for word with the text, the better was his recitation considered to be. For many institutions this was all. In the better institutions or with the better teachers specimens were brought into class or the students were directed to hunt up their own material illustrative of the lesson in question. Professor Prentiss, at Michigan Agricultural College, used the latter method, but it had its disadvantages, for the lazy student was satisfied to get by without hunting up and studying his own specimens. Here at Iowa State Agricultural College my father chose the other method. Thus, in his own copy of Gray's Lessons I find a slip of paper with the lessons indicated for April. The year is not given, but from other marks in the book may have been any time from 1870 to 1875. On this card is written for April 9, "Flowers of Maple. Consult such lessons as are necessary. Bring spms (specimens) to class." A similar note occurs for April 13, when the flowers of elm were to be studied.

Dr. Gray taught his more advanced course, for which his Botanical Text Book was used, mainly by means of lectures. He gave over his undergraduate teaching early in the seventies to Dr. Goodale, but in my

father's copy of the Text Book are his notes of a "Lecture to Junior Class of Harvard University. By Dr. Gray" for January 13, 1873. This book was taught without set laboratory work. Occasionally Dr. Gray would invite a few students into his private laboratory and show them some of the structural details under the (borrowed) microscope. Thus Dr. W. J. Beal, a student of his at intervals during the period of 1862-1865, wrote in 1917 concerning that time at Harvard, "During one spring Dr. Gray met three of us for lessons in this text-book freely illustrated by fresh specimens. The botanical department at Harvard did not own a compound microscope; it had the use of a thousand dollar instrument belonging to Lowell Institute. A little crude work was done, such as viewing the streaming motion of granules of chlorophyll in leaf- sections of Valisneria, looking at grains of pollen sections of ovules, etc."

In addition to the Gray's Lessons or the less often used Botanical Text Book, the students in some colleges were required to make "herborisations," collecting and pressing plants and preparing a herbarium. This work, done outside of the class-room, was in reality a sort of laboratory work, but represented such work without supervision by the instructor. Perhaps this independence from an instructor's immediate presence made the work of preparing such a herbarium more valuable for the earnest student. I hate to think how the dependent student got others to do his work for him.

A study of the catalogues of several institutions shows many things of interest, such as the courses taught, the text-books used, the equipment, the hours of class work, etc.

At Harvard the catalogue for the year 1869-70 shows that there was but one member of the faculty who taught botany and that was Dr. Asa Gray, Fisher Professor of Natural History. He was supposed to have an assistant, but the latter's name does not appear upon the roster of the teaching force. In the next catalogue, for 1870-71, the assistant is listed as William Gilson Farlow, M. D., Assistant in Botany. Tradition has it that Dr. Farlow, with his diminutive size and squeaky voice, did not make a success of his teaching by the method described above and was about to resign in great mortification. He was persuaded to go abroad to Germany to study, working there under de Bary. Upon his return two or three years later, he was given charge of the work in "Cryptogamic Botany" at Bussey Institute as Assistant Professor of Botany. With his deeper training and the use of the modern laboratory method, and furthermore, the changed type of students taking his courses, he was successful from the first and became the leading teacher of the subject in America.

In 1869-70 two courses were offered in Botany as follows: "IV Botany. Professor Gray, or his Assistant, will give a course of practical lessons in Systematic Botany, twice a week, from the first of May to the close of the Term, to Scientific Students, properly prepared for it"—"Also, a course of instruction with a Text-book, in the elements of Structural and Systematic Botany; two lessons a week, throughout the Second Term"— "Text-Books. Gray's Botanical Text-Book, or Gray's First Lessons in Botany. Gray's Manual of the Botany of the Northern United States." In the catalogue for the year 1874-75 we find Dr. Farlow back and giving, at Bussey Institute, a course described as follows: "Vegetable anatomy particularly the microscopic study of woods. Rudiments of crytogamic bot-

any. Fungi, especially those injurious to vegetation. Special investigations on the diseases of plants will be pursued." At Cambridge at the Botanical Garden botanical instruction for undergraduates was now given by Dr. G. L. Goodale for the first course, Natural History 2 and by Dr. Goodale and Dr. Farlow for Natural History 8, Advanced Botany. This class met twice a week, but no special laboratory period is yet mentioned, although the catalogue states concerning the equipment for the botanical teaching that "The Botanical Department has a thoroughly furnished laboratory, garden and greenhouse and its library and herbarium are the largest in America."

Not until the catalogue of 1875-76 do we find a definite period set aside for laboratory work, and that for "Natural History 8, Advanced Botany (Lectures and Laboratory Practice). Three hours a week.—Students in this Elective may, at their option, pursue the study of Cryptogamic Botany with Assistant Professor Farlow." In the three-year course in Natural History in Lawrence Scientific School we find it listed among the First Year subjects "Botany." Two lectures and two hours laboratory practice each week. Assistant Professor Goodale," and for the second year, "Botany. Two lectures and three hours of laboratory practice each week, Asst. Prof. Goodale," with a foot note indicating that "A lecture is counted as an hour of laboratory work. A recitation, for which a lesson is prepared, counts for three hours laboratory work. From each student forty-five hours work in all is expected each week." In the third year the course in Advanced Botany, under Assistant Professor Goodale required "nine hours of laboratory practice each week."

Thus, we see that not until 1875-76 did the botanical laboratory receive full recognition at Harvard, although its beginnings there are much earlier.

In 1867-68, at the only other university in the country, with a professor occupying a chair for botany alone, Yale University, this subject was not taught to the regular four-year collegiate students, but to the students in the three-year course in Sheffield Scientific School. There, in the third (Spring) term a course was given in which the text book was Gray's Lessons. In the course in Natural History and Geology, in this school, Professor D. C. Eaton, the Professor of Botany, gave the following courses in the winter term, "Botany. Lectures. Gray's Text Book." and in the spring term, "Botany. Gray's Text Book. Excursions and practical instruction. Gray's Manual"—"In addition to the regular courses of lectures on structural and systematic Zoology, and on practical Entomology and other special subjects, students are taught to prepare, arrange, and identify various collections, to make dissections and pursue original investigations.—In this section Botany may be made the principal study in place of Zoology, at the option of the student."

In 1868-69 the work in Botany for the Juniors and Seniors in the course in Natural History and Geology was outlined as follows:

Junior Year. 1st Term. Botany—Gray's Text Book. Use of microscope.
               2nd Term. Botany—Gray's Text Book.
               3rd Term. Botany—Excursions. Practical Exercises. Gray's Manual.
Senior Year. 1st Term. Botany—Excursions. Herbarium Studies.

> 2nd Term. Botany—Herbarium studies. Botanical Litera-
>   ture. Essays in Descriptive Botany.
> 3rd Term. Botany—Continued, with Excursions.

The 1876 catalogue gave the same lay-out for Botany as the fore-going.

Except for the mention of "Use of the Microscope" there is little to indicate that any laboratory work was carried on in connection with the text book, aside from the emphasis placed on excursions, and herbarium studies. At least it was not such laboratory work as we now require.

At the University of Michigan during the period of 1869-1871 Dr. Alexander Winchell was Professor of Geology, Zoology and Botany. At this time he had no assistant for his teaching work although Mark W. Harrington was assistant curator of the Museum of Geology, Zoology and Botany. He later became head of the Department of Botany. The Freshman in the Scientific Course was given a six weeks course in the second semester, one hour each day. This course is described as follows. "The elements of structural, physiological and systematic *Botany* are imparted in a course of familiar lectures and practical studies with specimens in hand, under the direction of the Professor. Text Book, Gray's Lessons"— "Advanced students will be furnished with the requisite facilities and instruction during the second semester.

Evidently Professor Winchell required the students to see the plants about which they were studying, and doubtless flowers were dissected and plants identified in the class-room as directed in lessons 30 to 32 of the text used. The laboratory idea was there in embryo, at least.

At Illinois Industrial University, now the University of Illinois, we find J. W. Powell, M. A., as Professor of Natural History and Geology in 1869, with Thos. J. Burrill as Assistant Professor of Natural History. Two years later the catalogue shows Powell gone and Burrill as Professor of Botany and Horticulture, and E. D. Hill, M. D., as Non-resident Lecturer on Vegetable Physiology and Fruit Growing.

Botany was offered in two one-hour lectures a week for two terms, while in a third term it was given as "Practical collection and examination of the flowering and flowerless plants from all parts of the State as far as possible. Botanical excursions and surveys." In the schedule of classes included in the catalogue for these years no laboratory work is shown for botany although in the Junior year the third year students of chemistry were scheduled as follows: "Practical Chemistry. 9-3: 30." The catalogue description of the course in botany for the first term is practically a table of contents for the text used, Gray's Botanical Text-Book. There is a significant statement, which I have placed in italics, in the description of the second term's work. "Systematic Botany in lectures: 1st, the natural orders, their extent, properties, uses and distribution, 2d, *use of the microscope*. Vegetable Physiology, continued. Chemistry of plants and of their food. Fungi and vegetable diseases, and outlines of the classes. Distribution and reproduction of the Cryptogamia. Two lectures a week."

The underlined statements I cannot interpret otherwies than that in the one hour recitation periods the students saw for themselves some of the objects they were studying. Clearly Professor Burrill was beginning to use laboratory methods sixty-five years ago.

Here at Ames my father was, I believe, the first professor of botany. To be more accurate, he came as Instructor in Botany and Horticulture, in February, 1870, becoming Adjunct Professor of these subjects in 1871 and Professor in November, 1872. In November, 1873, all chairs were declared vacant and the faculty was reorganized with my father appointed Professor of Botany, Zoology, Horticulture and Pomology. This settee was declined and he was made Professor of Botany and Zoology, the Horticulture being given to J. L. Budd. The entrance requirements here, as in most other small colleges of the time, were scarcely higher than those required now for entrance to a senior high school. Following the practice of those days the Freshmen were given a spring term course using Gray's Lessons, passing out to the class specimens illustrating the subject of the day's lesson. The Juniors were taught from Gray's Botanical Text Book, also with specimens to examine. No separation of laboratory work from recitation was made, according to a letter I have received from Dr. J. C. Arthur, of the class graduating November, 1872, during his undergraduate course, although the use of the microscope was a definite part of the work as early as 1871. It was apparently in the beginning of 1873 that my father startled the other members of the faculty by putting up the sign, "Botanical Laboratory," on the door of a small room cut off from the south end of the main hall on the second or third floor of the old main building. This laboratory was provided with one microscope, a table, jars of material preserved in alcohol, scalpels, needles, razor, etc. Here was studied the structure of higher plants, and the lower plants came under the eyes of the eager students.

The botanical activity in Germany under Hoffmeister, Sachs, de Bary and others had received little attention in this country for the reason that few botanists read German, and books and periodicals from that country were difficult to obtain, with the very slim budgets available. My father began to get some of these, including Sachs' Lehrbuch, and various other publications. These revealed to him the one-sidedness of the current botanical instruction. He never went to the extreme followed by the next generation of botanists, of discarding all of the old courses. He insisted, however, that they should form only a part, not the whole of the subject.

What the botany at Ames was in 1877 is well shown by a fortunately preserved letter of his to Dr. W. J. Beal, at Michigan Agricultural College, who evidently made inquiry about laboratory work in botany. From this letter, dated December 31, 1877, I quote the following: "I answer most emphatically *yes*. A college which proposes to keep up with the current must provide Botanical and Zoological laboratories. The college which does not provide such laboratories will fall behind the progressive institution, at least so far as the biological sciences are concerned. A botanical laboratory is just as necessary for the proper teaching of botany as is a chemical laboratory for chemistry.

"Well, now, as to plans. I will take the arrangement as I have it here at Ames.

1. FRESHMEN, 2d half yr. Elementary Botany, with study of crude (exterior) anatomy of plants. Class meets twice a week. No special laboratory *necessary* although one could be used.

2. SOPHOMORES, 1st half yr. Structure and classification. Analysis of plants: collection, and making of herbarium. Class meets twice a week. Should have a laboratory with tables. Will get as soon as I can 'make it,' a room large enough to accommodate all the class (about 50) at once.

Every student *now* supplies himself with a simple brass three-legged microscope which answers very well for a dissecting instrument. (Cost 75 cts. Magnifying power 9 diam.) Also with needle points and knife.

When I get my laboratory it is my purpose to have each pupil supply his own microscope, like the above, but we *may* provide scalpels and fine forceps.

3. SOPHOMORES, 2d half year. Economic Botany. Class meets twice a week. They also continue to analyze and classify. When I get my laboratory I will put them into it. Now I use class room.

4. JUNIORS, 1st half year, 4 times a week. Beg. Physiology & Crypt. Bot. All spent one afternoon a week (3 hours) in the Physiological Lab., which is fitted up with tables and microscopes for ten students. I use now ordinary tables, and provide for each pupil: One Compound Microscope 1 inch and $\frac{1}{4}$ in. obj., one good scalpel, one pr, fine forceps. Needle points. Reagents.

"The whole outfit for each student costs from 50 to 60 dollars. On shelves in the lab. I keep the material to be used and such extra reagents as are only occasionally used. I preserve specimens for examination in alcohol, and find them perfectly satisfactory.

"For ordinary tissues I know of nothing better than pumpkin vine taken at maturity—though still green. For the study of protoplasm I use young roots (2 or 3 days old) of Indian Corn. For pollen—mother-cells, and the development of pollen grains I use young flower buds of Lilac.

"Pardon my long letter, and believe me,

Yours very truly,

"C. E. Bessey."

"Beck's *Economic Microscope* does very well for a laboratory instrument where cheapness is absolutely necessary.

"I bought five for about $35.00 apiece by getting them *duty free*. They will cost less now. Hereafter I will buy a better grade of instruments."

What have we learned as to the old teaching? Some of it was good, some was poor. Under some teachers laboratory methods were used in part, under others nothing of the kind existed. The subject matter was one-sided because of the great need for Systematic Botany at that period of our history. I fear we may have become as one-sided in some institutions under the later methods of teaching. We must not be afraid to adopt new methods of teaching and new subject matter, but let us not forget that the older generation knew its field as few of us now know it, and that we should preserve that which was good of the old.

THE EVOLUTION AND DIFFERENTIATION OF LABORATORY TEACHING IN THE BOTANICAL SCIENCES

R. J. POOL

From the Department of Botany, University of Nebraska

We are assembled today in commemoration of the botanical achievements of one of the most significant periods in the long and fitful development of science. Meditation on these exercises has filled us with a thrilling sense of having participated, albeit in a most humble degree, in the great events in question. We have had communion with those great men, the beginnings of whose scientific careers coincided so closely with the initiation of this important phase in the history of our subject. The vast development of the various ramifications of science during the period has introduced relationships and problems in connection with the social, economic, and moral catastrophies of the day that seem to threaten the very existence of modern civilization itself. It is inspiring, indeed, that we are permitted to participate in these symposia within the very locality in which certain of the important foundation stones of our science were laid.

Modern science was born when the ecclesiastical universalism of the Middle Ages began to wane during the seventeenth and eighteenth centuries. Savants of the various European nations began to visit each other and to exchange ideas and opinions as to the nature of the universe without great danger of losing their jobs or their heads. The French were outstanding leaders in this important movement. Science came to "permeate literature" largely through the influence of the French Academie. The methods of exact science appeared considerably later in the great universities of Germany, England, and Italy. Astounding discoveries were being made, but the isolated and more or less trembling investigators themselves hardly sensed the full meaning of their findings.

The fore part of the nineteenth century saw the establishment of a few great scientific laboratories, mostly of physics and chemistry. The Cavendish laboratory at Cambridge was not founded until about the middle of the century when the influence of such men as J. J. Thomson, Lord Rayleigh, and Clerk Maxwell became infectious. But the laboratories were still almost exclusively the private and more or less mysterious workshops of the individual investigator. Nevertheless science had grown by this time to such a degree that certain "science," notably mathematics, physics, chemistry, and biology were clearly differentiated. Laboratory buildings soon began to appear on the quadrangles of a few of the greater universities of Europe and America. Limited opportunities for laboratory study, especially in physics and chemistry, slowly became available to immature or undergraduate students. The expansion of laboratory facilities for the study of plant science lagged, however, for many years, both in Europe and America. Botany was studied in college almost solely in connection with the preparation for a career in medicine. One of the greatest botanists of far flung international standing, de Bary, took his degree in medicine in 1853.

A noteworthy change with reference to botany and botanical teaching made its appearance in the late 1860's and the early 1870's in Europe, especially in Germany. Farlow wrote of going to Strasburg in 1872 to study in de Bary's laboratory, and finding that he was the only botany student there at the time who had studied medicine. The other students had begun their special botanical work in their first year in the university, and they had had considerable preparatory training in the subject.

The study of botany in the colleges and universities of the United States began a period of rapid development in the 1870's. Previous to that time there were very few professorships of botany in this country. True, Gray was at Harvard, Torrey was an active botanist, but chemistry was his real business, Eaton was at Yale, Porter at Lafayette, and Prentiss at Michigan State College and Cornell. Farlow came back from de Bary's laboratory with great mycological zeal, but the other men were interested mostly in taxonomy. A short time later that classic list was somewhat enlarged by the addition of Beal at Michigan State, Vasey, U. S. D. A., Brewer (although not primarily a botanist) at Yale, Bessey at Iowa State, Rothrock at Pennsylvania, and Burrill at Illinois. The many other great names which we associate with the three score years under review were written into history at later dates.

With the entry of these men into their professional work there appeared an awakening among the administrators of American colleges and universities to the thought that botanical science probably had something to offer for the development of the Republic and that it should, therefore, be encouraged. Charles E. Bessey completed his undergraduate work at Michigan State College at the beginning of this most interesting period. Scarcely had he finished his collegiate work at East Lansing when he was elected instructor in botany and horticulture, and secretary of the faculty at Iowa State College at a salary of $1,200. That was in February, 1870. Bessey met Asa Gray at the Dubuque meeting of the American Association for the Advancement of Science in August, 1872. Plans were made at that time for him to spend the following winter (three months) in study with Gray at Harvard. It is probable that Bessey learned much that winter concerning the trend of botanical work and the development of laboratory methods in Europe from Gray and Farlow, both of whom had lately returned from abroad.

From the standpoint of local history it is most interesting to note on pages 6 and 7 of the Fourth Biennial Report of the Board of Trustees of Iowa State College for 1871, that there were at that time—Bessey's first year there—thirteen persons on the faculty of the college. These included the farm superintendent, preceptress, teacher of piano music, matron, and a lecturer on bee culture! On page 45 of the same report we learn that: "The course on Botany occupies one year and a half, extending throughout the whole of the Sophomore year, and one-half of the Junior year. During the first year of the course the students acquire a knowldge of the principles of Structural Botany from the study of 'Gray's Lessons,' as well as by actual dissection and analysis of plants. Systematic Botany is taken up as soon as the student is far enough advanced to do so, and carried through the year, each student being required to collect, press, mount, and name at least one hundred species of plants."

In the Junior year Vegetable Physiology, Economic Botany, and the

Elements of Cryptogamic Botany are presented in succession, about an equal time being devoted to each. In the illustration of the subject, the College Herbarium affords examples of the more rare forms, while for minute structure a good microscope is in daily use. That, ladies and gentlemen, is the complete description of the very first botanical instruction offered by the department of botany in this (Iowa State College) institution. Thus we have the simple account of the foundation of what was probably the first botanical department west of the Mississippi.

Bessey used to tell me how excited he was when the regular school year opened on this campus in February, 1871, and he introduced his class to laboratory work in botany. The laboratory was a small room at the end of a corridor in the old main building. A label, "Botanical Laboratory," nailed on the outside of the door to that room, is said to have stimulated unusual emotions on the campus. The equipment consisted of rough board tables, a single compound microscope, for which the college paid $1,200, and a few reagents on shelves. This was the nature of the first formal botanical laboratory for undergraduate students in America. Botany was already being taught in several institutions, but no regular laboratory work had been arranged for undergraduates. The botanical laboratory at Harvard had been used for graduate instruction a year or so earlier, much after the custom in Europe.

One reads with much interest, in the subsequent official reports of the college, of the expansion of the courses and equipment of the department. Laboratory work and the use of the highly prized microscope soon became well established. Graduate courses in "Physiological Botany" and "Systematic Botany" were established in 1876, and at that time there were "seven compound microscopes with Tolles' and Beck's objectives" in use, and "acoholic and dry material for examination" in the botanical laboratory. The Report of the Trustees for 1879 states that: "The Department of Botany and Veterinary Science are located in a handsome brick building in the Italian Style. On the first floor is the Botanical Laboratory, Lecture Room and the Professor's Room."

By 1880 there were eleven compound microscopes with Hartnock's, Tolles', and Beck's objectives. The "rudiments of medical botany" also appeared in the description of the courses offered for that year. The Report for 1883 states that the botanical equipment consisted of: (1) A laboratory capable of accommodating twenty students at once; (2) twenty-one laboratory microscopes, with inch, half-inch, and sixth-inch objectives made by Tolles, Harnock, and Beck; (3) one Beck's first-class binocular microscope, with accessory apparatus, and high power objectives. The official announcement of the college for the year 1885 lists Dr. Bessey as Vice-President and states that the college herbarium contained 14,000 specimens, and that the department was equipped with a good botanical library.

Bessey wrote to Beal, at Michigan State, in December, 1877: "A college which proposes to keep up with the current must provide botanical and zoological laboratories. The college which does not provide such laboratories will fall behind the progressive institutions, at least so far as the biological sciences are concerned. A botanical laboratory is just as necessary for the proper teaching of botany as is a chemical laboratory for chemistry."

During Dr. Bessey's years at Iowa State he also introduced laboratory work in botany into the universities of Minnesota and California in connection with courses which he gave in those young institutions while he was on vacation from Iowa. He often spoke in great glee of his experience in "carrying microscopes to Minnesota."

Not only did Bessey introduce the technic of the botanical laboratory into undergraduate courses in plant anatomy here at Ames; he also gave instruction in plant physiology and plant pathology in which microscopes and laboratory methods in general were regularly employed. His junior students in physiology and cryptogamic botany spent one afternoon a week in the physiological laboratory, which was fitted up with tables and microscopes for ten students. His interests in phytopathology, which was then unorganized for teaching purposes, is clearly indicated by the inclusion of special instruction in rusts, smuts, molds, and mildews. Plant pathology also was taught incidentally with botany by Burrill at Illinois in 1873, and was taught first as a special subject by Farlow at Harvard in 1875. I am unaware of the use of the use of the laboratory in these cases. The first distinct department of plant pathology was established at Cornell in 1907. The Seventh Biennial Report of the Iowa State Agricultural College and Farm for 1876-1877 includes Bessey's paper "On Injurious Fungi," which is an excellent treatise on the "Blights or Erysiphei." This contribution is accompanied by two plates of thirty-four original figures drawn directly upon stone by the aid of the camera lucida.

One of the most important of Bessey's contributions to the teaching and development of the laboratory method in botany while he was at Iowa State was the publication on April 12, 1880, of his "Botany for High Schools and Colleges." This well known text made available for the first time to young American botanists much of the material that students on the continent had enjoyed for several years in the classic *Lehrbuch* of Sachs, the *Vergleichende Anatomie* of de Bary, the *Traite General* of La Maout and Decaisne, and the *Genera Plantarum* of Bentham and Hooker. The preface to that book states that: "For the student who desires to pursue the subject further, or who intends to make botany a special study, this book aims to lead him to become himself an observer and investigator, and thus to obtain at first hand his knowledge of the anatomy and physiology of plants; accordingly the presentation of the matter has been made such as to fit the book for constant use in the laboratory, the text supplying the outline sketch, which may be filled up by each student, with the aid of the scalpel and compound microscope." Numerous suggestions for specific laboratory exercises are noted throughout the book. This publication, plus the "Briefer Course," which went through several editions, left an indelible stamp upon the development of botany during the period involved in our celebration. These two books helped mightily to mould the progress of laboratory teaching of botany in the colleges and high schools for two score years.

Bessey went to Lincoln in August, 1884, to become the first professor of botany and dean of the Industrial College at the University of Nebraska. He founded the department of botany there and began building its future in the light of the valuable experience he had had at Iowa State. All of the science work at Nebraska had been handled under a department of "Natural Science" with a single man in charge previous to that time.

The catalog of the University of Nebraska for 1884-1885 records the beginnings of the new department and we find there the statement that: "Throughout the course the student makes investigations in the laboratory and field. The laboratory has a good outfit of working apparatus, including six new microscopes lately purchased." The courses offered that year were: Vegetable Anatomy, Vegetable Physiology, Special Anatomy and Physiology of Lower Plants, and Special Anatomy and Physiology of Higher Plants. Graduate work in botany also was announced. The program for the new department for 1886 included Vegetable Anatomy, with five hours laboratory, Vegetable Physiology with five hours laboratory, and Special Anatomy and Physiology of Lower and Higher Plants each with five hours laboratory. A special advanced course involving a study of the Physiology of one plant was added in 1887, and Comparative Anatiors. Studies in the Fertilization and Propagation of Plants, Hybridizaomy of Particular Tissues and Organs was available for Juniors and Seniors, Diseases of Plants, all with laboratory work, were included in 1888-1889, as were special courses on Variation in Plants, and Plant Food, all with laboratory work.

The old Industrial College Building, afterwards named Nebraska Hall, was built in 1888, and the botanical department was nicely housed on the first floor of that, the third building on the campus. A special laboratory for plant physiology was one of the significant features of that building, and the cornerstone bears the inscription, "Science with Practice," suggested by Dr. Bessey.

New departments of botany were now appearing in various institutions and the general introduction of laboratory teaching in botany became the established practice by this time. Other phases of our subject, such as histology and physiology, became represented in special courses in widely scattered departments. Advanced courses in physiology with regular laboratory work were added at Nebraska in 1890-91, and in 1897 we find a combined course in Vegetable Physiology and Pathology with laboratory and field work. Phytogeography, ecology, and cytology now made their appearance in the teaching program, and in 1899 advanced work in pathology, with laboratory studies, was included in the offerings of the department.

At the turn of the century, with widespread and intense interest in science centering in the colleges and universities, there came a rapid differentiation and specialization of botanical teaching. The next twenty years were marked by an expansion of personnel and material equipment for the teaching of botany which very soon outstripped the best that Europe could offer. Our botanical graduates found ample facilities for graduate work at home and it was comparatively easy to get a job upon receiving the degree.

The three phases of our work which forged far beyond the others in this rapid development were pathology, cytology, and genetics. Horticulture and bacteriology had already grown to the point that made it wise to create separate departments to handle their special interests. The expansion of the program of the United States Department of Agriculture and the various state Experiment Stations during this time called for many men trained along these lines. The intensely economic features of pathology and genetics stimulated the rapid growth of those fields. The rapidly

expanding interest in breeding and genetics served to force cytology to the front. There have been three strikingly modified editions of our best American textbook of cytology in the past thirteen years.

The details of the rest of the story are so numerous and so close to all of us that I need not bore you with their recital. The progressive evolution of our subject will continue, for there are many alluring problems which still face the special investigators in plant science. The technic of the laboratory has become the principal aid in the attack upon the highly involved questions that puzzle the twentieth century botanist.

But what should be the future objective of the teacher of botany and, indeed, of science in general? We are told by cultured and serious-minded leaders that civilization is in danger of complete disorganization and disintegration because of the advance in scientific learning. As long ago as 1897, Woodrow Wilson, in an address at Princeton, said that the "scientific spirit" was dangerous, was yielding questionable service, and was causing degeneracy in the nation. Of course, the learned Wilson, like so many critics, did not distinguish between the "scientific spirit" and the material results of the findings as they were being warped and moulded to fit the personal needs of a growing body of unscrupulous citizens. Still, that critical attitude toward science persisted and flourished. After forty years science is no longer novel, but nevertheless, our critics still regard it as a menace to the improvement of the quality of human values. Spokesmen are calling upon scientific men to declare a holiday for a decade or so in order that the race may catch up with its findings.

Educational leaders anxiously inquire into the causes of this unrest that is so apparent in the colleges and universities. Science teachers ask if science is really "doing the job" that it should do. There are those in high places who agree that we must compete with outside attractions, that the schools must include the movie and the talkie, if we are to "motivate" our work and keep the boys and girls in our classes. Must of this motivation tends to dilute and gloss over our courses of study and to lead to still further superficiality and to the very uncertain end products of the movie. What we need, more than anything else, from the kindergarten to the graduate school, is to get back to a basis which involves fundamentals and to forget about a lot of this twentieth century foam and fluff.

Herbert Spencer said that science is a mode of life. We teachers of science need to keep that Victorian slogan more constantly in view. Especially do we need to impress the significance of the Spencerism upon our good brethren of the social sciences. They freely admit that their special interests in society have not kept pace with the progress of science. That state of mind constitutes a most fertile feature of the academic environment at the present moment. Certain leaders are bold enough to suggest that we should try a few experiments in the field of a scientifically planned society. Let us try to lead these friends into a more nearly complete realization of just what the mood of the scientist and the technic of the laboratory may do for them in their search for the truth that will lead them out of the complex social and economic mess that has blasted civilization in this most enlightened age of its evolution.

Even the most widely educated and cultured nations are not yet characterized by a citizenry that is *science conscious*. The essence of science is still the possession of comparatively few. It is extremely dis-

tressing to note, in the face of this situation, the growing tendency to minimize the role of science in daily life and to reduce the time allotted to science teaching in our schools and colleges. The time allowed for laboratory work in schools of all grades has been steadily decreased in the past two decades. This is certainly to be desired if it is demonstrated that science teachers have so miserably failed in the transmission of a proper sense of the fundamental values of basic science to the coming generations. Perhaps we have all been too intent upon the implantation of the special facts and features of our own particular phases of science into the lives of youth that we have blundered past the deeper and the more valuable generalizations. We may have failed to present those lessons of science that are tremendously valuable in the daily life of all citizens. Perhaps we should strive to develop a race of botany teachers who are able to move far beyond their own personal specialties and tap the realm of fundamentals for use in a type of inspirational instruction that would not only spread the good tidings of science but would go far toward developing a scientific consciousness at large. Perhaps we need more teachers who possess the personal charm and the tremendous scientific power of the masters of the old natural history period which gave way to the intensive specialization and differentiation that has marked the epoch which we commemorate today.

We have not always been careful to demonstrate to our students just how the scientific spirit and the scientific method may serve to guide them into a more complete, enjoyable, and beneficial life, no matter if they never go beyond our introductory courses in their formal college career. We have tended to present too much specialized science. Perhaps we have used the scalpel, balance, and the microscope too much or too constantly for the most good to our students. Perhaps we have thought too much of our subject and too little of the student. We may have taught botany too much and the student too little.

We cannot consent, however, to the elimination of all demands upon the individual student for the masterful accomplishment of a certain amount of work in our laboratories. Students too readily incline to treat their classroom obligations by the same code of deteriorating personal responsibility that governs their patronage of the movies. Tardiness, slovenliness and vulgarity are no longer signs of deteriorating personal habits, they are to be praised. Absence from classroom exercises is officially recognized and is expected in many schools and departments. A growing lack of attention to one's school obligations, to one's social, moral, and religious growth, to one's health, and general responsibility to home and society seem to mark human life today. Students do not like to be "tied down" to the performance of a certain task, in a certain way, and at a certain time. This is one reason why they do not enjoy laboratory work. And yet Huxley said that: "The best way to learn how to do a thing is by doing something as near like it as possible." If one can learn to do a thing "under easier and simpler conditions" so much the better, but that comes, as a rule, only with nerve-racking application and with the refinement of accomplishment from day to day.

No worthwhile intellectual discipline has been or ever will be mastered without a copious and constant application of toil, trouble, and tears. We need the science of classroom and laboratory more than ever in order

that we may approach life's problems more intelligently. If science is properly taught it will confer upon our civilization a broader skill to distinguish between what is correct and what is incorrect, what is right and what is wrong. Above all else, it should teach that all men are *not* created equal, that they are *not* equal, that "one opinion may *not* be as good as another."

Happy, indeed, is the boy or girl, the man or woman whose intellectual, social, and moral ideas become elevated by an adequate comprehension of the mood and method of science as they search for the truth that will enable them to live a more nearly normal life under the impartial controls of natural law. It is a constantly thrilling experience in the life of good teachers in our colleges to utilize the lavish resources of the modern laboratories of science to mould lives of that sort. Karl Compton wrote within the month that: "No other organizations have such a continual supply of new blood and new ideas, brought in by the ambitious, exuberant youth in staff and student body. They have a great tradition of intellectual activity and freedom, from which springs originality." A vastly more beautiful civilization is assured for the future if we teachers of botany add to our technical accomplishments the universal recognition of science as the proper instrument for the intelligent planning of the social, economic, and moral structures of the race.

THE PLACE OF BOTANY IN A LIBERAL EDUCATION

Edmund W. Sinnott

From the Department of Botany, Barnard College, Columbia University

"The study of Botany," wrote the estimable Mrs. Lincoln more than a century ago, "seems peculiarly adapted to females; the objects of its investigation are beautiful and delicate; its pursuits, leading to exercise in the open air, are conducive to health and cheerfulness. It is not a sedentary study which can be acquired in the library."

The picture of our science thus so charmingly set forth by one who did much to establish a place for it in the educational system of her day is probably not greatly different, one must regretfully admit, from that which still exists in the minds of the majority of people. However earnestly we may protest that botanical science comprises much more than this and however persistently we may practice and teach the art of the test-tube and microscope in the study of plants, our profession, in the minds of many, still has its chief goal a knowledge of the names and haunts of "the flowers that bloom in the spring." Would that there were a biological New Deal which should abolish the associations of the past and start us all off afresh as "phytologists!"

Of course everyone whose opinion is of value recognizes the honorable position which botany has attained among the sciences, because of the variety and importance of the contributions which it is making to the store of human knowledge and the immense practical significance of its discoveries. No doubt can exist as to the necessary position which a study of plants must always occupy wherever scientific research is maintained. The role which botanical science should play in education, however, is a question of more difficulty. It must obviously have a part on the program of every university in order that a supply of trained investigators and specialists may be maintained, but its particular function in the undergraduate college has not been always clearly understood. In those institutions where a vocational interest in a knowledge of botany exists, as notably in colleges of agriculture, this question does not arise, for the essential position of our science in the curriculum is universally recognized. It is rather in the colleges of liberal arts, or in general courses at other institutions, where the cultural rather than the vocational importance of a subject determines its value, that the question presents itself most acutely. What place, we may ask ourselves, should the science of botany occupy in a so-called liberal education? What contribution has it to make toward that goal of all our efforts, the development of truly wise and cultivated men and women? This continues to be the major problem which confronts those who are responsible for the teaching of botany in our American colleges today, and our answer to it must determine very largely what shall be the content of our botanical courses, especially the elementary ones, and what methods of instruction we had best employ in them.

Aside from its important role, which it shares with the other sciences, in developing a truly critical and scientific attitude of mind, there are at least three more specific major contributions, it seems to me, which botany makes to a liberal education; and these, in their cumulative importance, are so significant that they should elevate our science to a much higher place on the college program than has heretofore been assigned to it by curriculum-makers.

First, the type of botancial erudition which Mrs. Lincoln describes and which to our grandfathers constituted botanical science should by no means be undervalued. A wide acquaintance with plants and the ability to determine their names and relationships are by no means inconsiderable or useless achievements. In these days when the motor car has made the delights of a wide range of countryside and wilderness accessible to almost everybody, surely one of the most important satisfactions which we can attain consists in a familiarity with the diverse plant population which surrounds us. An excellent way of answering that almost universal human urge to collect something is to do this in the good old botanical fashion, with vasculum, trowel, and press. Such an avocation is not only a delight to him who indulges in it but often contributes substantially to scientific knowledge. For those whose most intimate contact with plants lies rather in the garden than in the field—and their rapidly growing numbers are one of the significant signs of the times for botanical science—a familiarity with the names and the classification of plants is also of the utmost value. We therefore need not apologize for the contributions which the botany of our grandfathers is capable of making to the life of today. In the education of many it will continue to occupy an honorable place. It must remain, nevertheless, the prerogative of those who love it rather than a necessity for everybody—one of the dessert courses on the educational bill of fare. No one proposes to make systematic botany a requirement for the bachelor's degree.

A far more important educational contribution of botanical science, however, and one which is most commonly cited as the chief justification for the inclusion of botany in the college curriculum, is the clearer understanding which it provides of so many factors in the environment of civilized man and the more intelligent control of these factors which it therefore makes possible. Surely such training must be one of the aims of all sound education.

The practical advantages of this sort which flow from a knowledge of botany are diverse and familiar. Thus in our complex industrial civilization, which is necessarily based on an abundant supply of energy, how essential it is for everyone to realize that all but a relatively small part of the energy which runs our machines was made available by the photosynthetic activity of green plants in the past. A knowledge of this fact is a necessary basis for comprehending the problem of conservation of our natural resources of power; and a recognition of the unique ability of green plants to convert kinetic energy into potential form serves to emphasize the further possibilities in this field which are now attracting so much attention, and the desirability, if our type of civilization is to survive, of doing for ourselves in the factory what green plants have heretofore done for us. The problems associated with that large portion of our population which dwells on the land are engrossing sociologists

and economists as never before; but underlying the whole question is the fundamental fact that agriculture itself is but the persistent care and nurture which must necessarily be lavished upon green plants if we are to live, for the same photosynthetic activity which has stored energy in coal and oil also furnishes all the fuel which runs the bodily machines of animals and men, and all the materials of which these machines are built. Surely there is no more fundamental fact with regard to the natural world than this relation of green plants to energy and to food, but its significance is by no means universally realized even by so-called educated men.

A recognition of this importance of plants demands an intelligent comprehension of the factors upon which their successful growth depends. To be awake to the peril of soil erosion one must know the absolutely essential part which the soil plays in plant life. The cause of "worn out" and impoverished soils, with all the human ills which follow them and the remedies which must be applied to restore them, can be fully understood only if we know the rôles which mineral salts and organic matter play in plant nutrition.

An acquaintance with destructive plants is also necessary. Most people know in a very general way what bacteria are, but an understanding of their growth and activity sufficiently intimate to combat them intelligently is rare, and a knowledge of other parasitic plants is almost entirely confined to specialists.

In addition to all this, a somewhat more specific knowledge of what is more narrowly called "economic botany"—the source and character of important plants and plant products such as rubber, cotton, timber and many others so essential in modern civilization—is highly desirable for everyone.

Because of the varied ways in which plants touch human life the vegetable kingdom has thus gained in recent years an importance which has even begun to be reflected in some of our political questions. Certainly no citizen is capable of making a wise decision with regard to these problems who is not conversant with at least the rudiments of plant structure and activity.

The practical value of a botanical education, however, goes much deeper than all this. Botany is an integral part of biology and a knowledge of it is essential in meeting all fundamental biological problems. Certain of these, particularly as they concern man himself, are among the most pressing which must be faced today. A knowledge of our physical environment is important, but an understanding of our human environment is even more so. Here lie those vital questions which we recognize at once as most urgent and serious. The theory of relativity or the conception of an expanding universe awaken nothing deeper than our intellectual interest, whereas the hypothesis of Nordic supremacy, the debate over birth control, or the assumption of a biological necessity for the class struggle—all of which are biological problems—plunge their protagonists at once into acrimonious discussion and may well imperil the peace of the world. Let us examine a few of these briefly to see what light even the very imperfect biological knowledge of today may throw upon them.

Race is merely an expression of the genetic variability of the human species. The problems of the origin of races, the inheritance of racial

traits, and the accomplishment of desirable changes in our human stocks can be approached intelligently only from a sound understanding of the laws of heredity and variation. The biological basis of sex is so obvious as to require no comment, but a sound knowledge of the origin and significance of sex is needful if one is to separate true science from pseudo-science in this vexed subject. A dogmatic application of Darwinian ideology to the control of human society is evidently premature so long as biologists themselves are still uncertain of its fundamental soundness in the rest of the organic world. In the field of human relations the vigorous attempts of politics, sociology, and economics to establish themselves as scientific disciplines have been in large part unsuccessful because of the many unknown variables in their material. Man is a conspicuously unsatisfactory laboratory organism, and a knowledge of human biology far more complete than we now possess and based on an understanding of all forms of life is needful before we can formulate with any degree of exactitude the laws which govern the behavior of that extraordinary species to which we belong.

The rôle of botany in these problems is not obvious, but it is nevertheless important. The life sciences cannot be segregated into two sharply distinct groups, for botany and zoology are complementary to each other; and to understand any living organism, even man himself, an acquaintance with organisms of all sorts is essential. The notable contributions which a study of plants has made to our knowledge of heredity and variation, from Mendel's day onward, are universally recognized. The biology of sex is far from complete unless it includes a knowledge of sexuality in those simple plant-like forms where it apparently first appeared. Even the complex psychological behavior of animals has its beginnings in the simple phenomena of stimulus and response which can be studied so directly in botanical material.

In view of all these facts it is therefore not too much to hope that the day will soon come when the intimate relations which exist between biology and human affairs shall receive their due recognition, and when at least a modicum of training in the life sciences shall be universally required for a college degree. In that more fortunate time it will no longer be possible to send forth into the world men and women to be engineers, politicians, economists or philosophers who have never seen the inside of a biological laboratory.

In presenting the claims of their science, however, botanists are too often inclined to stress these obvious practical advantages and to neglect a contribution of much greater ultimate significance which botany should be making to the life and thought of cultivated men and women. Education ought indeed to help a man to keep his body and soul pleasantly together and to dwell peacefully in a well constructed social order, but it should do much more for him than this. Pressing upon him from every direction are questions about himself, about the universe, and about his relations to it—questions which have no immediate practical importance but which continue to perplex him more and more as he grows in knowledge. Some sort of answer must be given to them, some sort of life philosophy erected, by everyone. What such a philosophy shall be is obviously of the utmost consequence not only for the individual but for the society in which he lives, since it ultimately expresses itself in his acts;

and one of the chief purposes of a liberal education is to provide an intelligent basis for the construction of a philosophy of life. It is here that biology assumes a major importance. The physical sciences indeed excite our admiration at the magnificent picture of a law-abiding universe which they present, but the picture lacks the warmth of life. The ultimate question which we ask of the universe, and the one in which are focussed all those which perplex us most, is not the nature of matter or energy or time, but something deeper than all of these. It may be stated very simply thus: What is the place and the significance of life in a lifeless universe? However gladly we may admit that the mathematician and the physicist and the philosopher may have a part in determining the answer to this question, we must maintain that it is primarily a biological one and must be approached from the basis of biological knowledge.

It may be objected that botany has little to do with philosophical matters of this sort, which, if within the domain of science at all, are problems for general biology and more especially zoology, to answer. It happens, nevertheless, that in the most important of such problems the contributions which botanical science can make may well be regarded as more valuable than those from any other source. Let us consider two or three instances.

Surely no question in this field is more fundamental than that of the origin of life. Here the botanical evidence is peculiarly important, for the first organisms were necessarily more like plants than like animals, since they must have been able to live without previously elaborated organic material. The simplest plants today are far more primitive than the simplest animals, and those remarkable bodies, the filterable viruses, in which many seek hopefully for a link between the organic and the inorganic worlds, are to be claimed by botany. If we are ever to gain an inside into the origin of life, it must apparently come through a study of plants.

Or shall we ask that ancient question as to what life itself really is? Such a complex phenomenon may be examined most helpfully both in its very highest and its very lowest manifestation. A study of animals and especially of man himself tells us much of the tremendous possibilities inherent in life, but the problem in its lowest terms can be approached only in plants. Plant protoplasm is one step nearer the inorganic than animal protoplasm. The basic syntheses are made in plant cells. It is here that the general properties and capacities of living stuff, not yet sharply segregated by organization and differentiation, can best be studied. It is little to be wondered at that physiologists are paying respectful attention today to plants in their search for the ultimate nature of protoplasm.

Or is it that tantalizing problem of individuality, of personality, which we seek to solve? Is each of us an extraordinarily complex colony of billions of protoplasmic individuals or is there some integration in us, some ego, perhaps even a "soul"? In its last analysis, this is little more than that old biological controversy between the Cell Theory and the Organismal Theory which is still one of the battle-grounds of the experimental morphologists. Nowhere, perhaps, can it be studied so fruitfully as among plants, with their loosely organized individuals, and especially among those lower forms where the organism is just emerging from the colony. Indeed, one might recommend to a student of the phylogeny of

souls that he begin his researches with a consideration of the lower green algae!

It is toward the solution of such fundamental problems as these that biology can make its most notable contribution to education. It cannot solve them all nor do more in many cases than to show how deep our ignorance is; but certainly the day is long past when the philosopher or the theologian can give intelligent consideration to his own problems unless he is acquainted with the fundamental facts already ascertained not only of biology in general but of botanical science in particular. Botanists have been too modest in assuming that their science has little to do with such matters. We should not hesitate to maintain, as is no less than the truth, that botany is one of the two or three basic disciplines upon which the entire body of human knowledge must rest. We may therefore conclude that, useful as botanical science may be as a pleasant accomplishment and indispensable as it undoubtedly is for an intelligent control of our environment, its most important rôle in man's education will come to be recognized as that of a notable guide and interpreter for the human mind as it gropes among the deepest problems of the universe.

If this evaluation is a sound one, college instruction in our science should evidently be so planned and conducted as to bring out most fully not only the practical value of botanical knowledge but these more general relationships to life and thought. This does not mean that we should fill our students with speculations in biological philosophy, of which there are perhaps already too many, or that we should pretend that a course or two in botany will enable them to solve many of the problems of the universe or to construct at once a satisfying philosophy of life; but it does mean that we should teach plants as living things rather than as names or pedigrees or structures alone. This suggests, to be sure, an increasingly physiological point of view in our work. Taxonomy and morphology are being revitalized at many points by physiological influences, nor can they continue to exist today as purely static sciences. We should ask ourselves whether there is not still too much emphasis, in our elementary courses, on phylogeny, classification, life histories, and the more descriptive types of morphology, and too little on energy relations, protoplasmic studies, reproduction, and morphogenesis. Upon a rich and rigorously taught factual basis in every field we should, of course, insist. If with all this, however, we can convince our students that every aspect of the study of plants bears some relation to the fact that plants are *alive*, and that knowledge pertaining to these remarkable living things may well touch ultimate problems far beyond the laboratory table, then our science will attain more fully the lofty place in education which it so richly deserves.

SOME PROBLEMS IN AN ELEMENTARY BOTANY COURSE

George S. Bryan

From the Department of Botany, University of Wisconsin

Every institution of higher learning, whether it be state supported or privately endowed, finds itself facing new conditions. The old order is changing, in some quarters slowly, in others more rapidly. Values are being called into question; are undergoing reappraisal. Quite naturally the questions and problems affecting universities as a whole are reflected in the courses offered at the institutions. All of us, I am sure, welcome this advent of discussion and criticism, especially if it takes constructive form. It is through such means that thought may be stimulated, objectives clarified, and perhaps the way paved for further growth and development. The changing order of things has reopened old, and brought into being new questions and problems concerning the elementary course in Botany. The nature of such problems and the extent to which each problem may affect the course will obviously vary in different institutions. However, I am hoping that we may find at least some common denominator for the questions and a common interest in them as we proceed with these remarks.

Some universities, especially those in which all schools and colleges of the institution are centered in one locality, have a particular problem in the wide diversity of interests and needs of the students entering the course. This year, for example, in a certain institution the elementary botany class is drawing students from the following schools and colleges: agriculture, applied arts, education, journalism, letters and sciences, pharmacy; and even engineering and the Graduate School are represented. It is obviously impossible to meet all the needs and interests of such a diverse group. If a single course is to be maintained its effectiveness can be greatly aided by friendly coöperation and friendly criticism arising from the schools and colleges involved; and in a leadership that makes an earnest effort to discover as many common denominator values as possible for all concerned.

I turn next to the relatively new problem—the large number of students in the course. Twenty or twenty-five years ago the number of students in the elementary botany class in many of our institutions was comparatively small. Today a very different situation commonly prevails. A mass is surging into our class rooms. This semester, for example, the elementary botany class in the institution which I represent has 464 students. I suspect other universities may show comparable or even larger numbers. Herein lies an evidence of the new era. Herein, also, lies the problem of how to work efficiently with these large numbers. In some institutions, at least, this influx has not been accompanied by a corresponding increase in experienced staff. In consequence there are large laboratories and crowded discussion sections. It will not do to pass this problem, with a shrug of the shoulders, over to administrative officers. They have to live in the practical world of budgetary limitations

and deficits. They owe us at least an understanding of our difficulties
and a willingness to cooperate in every way possible. But that, on the
other hand, does not free us from our obligation of pondering the problem,
of attempting to find effective means by which the objectives of the
course may be reached in spite of the large numbers.

There are the questions raised by the presence of what I may call
the "problem students" in our classes. They are the ones who are not
succeeding in the course. We are all only too familiar with the types:
the plain, downright dumb; the painfully slow; the openly indifferent; the
giddy light-weight whose chief concern lies in the due application of her
lip stick and powder puff; the play boy who looks upon the university
as a sort of glorified country club. What shall be our attitude toward
these individuals? In some institutions both administrative officers and
instructional staff spend much time laboring with these misfits. I often
wonder if we are justified in spending so much time and energy on these
individuals. Might not results more worth while be accomplished by
using this time in conferring with and encouraging the more educable
portion of the class? Here may be mentioned practices of advancing in
grades students who are unfitted, and in some high schools of awarding
diplomas to the obviously unfit.

Although the course in Botany is generally listed as a freshman sub-
ject there may be enrolled a considerable number of upper classmen.
Their presence brings about the question of segregation. Is it practicable
and even desirable that they be brought together in special sections?
There is also the allied question whether in the large group of freshmen
we shall establish special sections for the more able among them. The
problem of segregation has its pros and cons, its advantages and its dis-
advantages. I am rather inclined to the opinion that the advantages are
outweighed by the disadvantages. The fact that an individual is an upper
classman is no guarantee of superior capacity. After the course has gotten
under way it takes time to determine for each studetn the relationship
between capacity and performance. If selection and segregation of indi-
viduals into particular groups is to be made it should be attempted at the
beginning of the course. Such attempts at later times often involve ad-
ministrative difficulties to say nothing of the questionable advantages. I
rather think a sprinkling of superior students in any group may be helpful
to all concerned. They may act as yeast leavening dough. But under such
conditions the slower, duller end of the group must not be allowed to set
the pace for the progress of the group. If that is permitted the more able
members suffer. Classes in English and other languages have sections for
apt students. So far as results are concerned one may hear comments
both favorable and unfavorable.

And now I propose three questions or problems (if we desire to
call them such) about which we may have endless discussion, about
which there may be no unanimity of opinion.

I refer (1) to the question of the *contents* of the course—what shall
be the subject matter of the course; (2) what *methods* shall we employ;
(3) what are the *objectives* of the course? And by this I mean what are
we trying to do for the student? *Contents, methods, objectives*, these
three, but the greatest of these is *objectives*.

The elementary botany course at our institution is the product of a slow evolutionary process. It was begun by others, it has passed through the moulding hands of many. Nor is that all, for each course reflects not merely the work and the thought of many men, but also the circumstances, the factors of the environment under which it came into being and under which it has grown. A course at a given institution may thus acquire what I shall call its own distinctive flavor.

In such a process of evolution I think we must be careful to see that in regard to *contents* and *methods* we do not lose sight of *objectives*. Frankly, while I am interested and concerned as to *what* we teach and the *hows* we employ in the process, I am vastly more concerned in attempting to clarify for myself the objectives of the course. By that compass we may chart our way.

One of the first questions to be asked respecting the course is this: (and I ask it, not perched up in the clouds of educational theory, not seeking vague generalizations, but with feet firmly planted on the ground). What objectives have we set for the course? What do we hope to have our students gain thereby? Let me begin by suggesting that our educational processes do not, cannot *create*. The most that these processes can accomplish, all any course can do is to stimulate the development of those characteristics the genes of which are possessed by the individual. It seems to me we cannot overlook this most evident objective—that of attempting to *stimulate* the *development* of characteristics. It is possible to be still more concrete. We live in a *scientific* age; we are studying a *science*. Surely it is not too much to ask that our students be led to appreciate something of the *spirit* and *method* of science.

I would attempt to stimulate in my students the spirit of inquiry which leads to observation and experimentation and to reasoning therefrom. It is the spirit that is content not with the superficial, with mere *words*, but which seeks to *understand* as far as possible. It is the spirit that develops the *critical* attitude of mind. Surely these are worth while, concrete objectives. I would have my student experience the laboratory method. As I have implied above, it is the method of observation and experimentation and of reasoning therefrom. I would have him *see* and *do* as much as possible for himself, for I am fairly confident of this: That real growth comes in terms primarily of what the individual does for himself. Said a very able and distinguished member of the Federal Trade Commission speaking of university training, "It is not so much *what* you take as *how* you take it." And he added this prescription, (speaking to the students) "You work!" I see little chance of developing the valuable qualities of initiative and resourcefulness in individuals by treating them as little pitchers to be poured into.

I have been dealing with the aspects of trying to stimulate the development of characteristics. In doing so I would not have you think for a moment that I am implying that the course is intended to train botanists. In the past that may have been regarded as one of the chief objectives. Today I am sure we are taking a broader view. We are seeking not only the development of the individual; but such aspects as the broadening of horizons, the stimulation of an interest in nature, and, perhaps, even more, of having the student see the place of science and its limitations.

CONTENT OF GENERAL BOTANY COURSES

Homer C. Sampson

From the Department of Botany, Ohio State University

Some of the problems involved in supplying students in the general botany course with adequate, critically selected, and readily obtainable data in commendable form seem to me to be the most urgent at the present time. When the bases and potential values of the general course were more limited than at present perhaps there was some excuse for making the finding and recording of facts the central theme. But we are now facing a very different situation.

During the present century we have seen general botany pass from a study of one or two phases of the subject to a study of its many phases. This alteration may be ascribed to new discoveries in botany and to the broader training, enrichment of perspectives, and broader social aims of the instructor. We have seen the fields of plant pathology, physiology, ecology, cytology, genetics, bacteriology, and various phases of applied botany developed to the point where we are now supplied with many fundamental facts and ideas of value to citizens in every walk of life. The problem we are facing is not the question of whether we should select and incorporate these facts and ideas in the general course, but whether we are incorporating them in form only, or in a manner that functions. One of our senators said of a certain aspirant to a minor office, "He has the information but it doesn't functuate." Last week one of my colleagues said of himself, "Really, I did not begin to teach until I had mastered the subject matter and its implications sufficiently well that I could forget about it and devote my attention to the reactions and progress of the students."

During the past two decades the number of students in our classes has increased many fold. These students come from a great variety of home environments, and have a great diversity of interests in life. On the campus and elsewhere they are meeting an ever increasing variety of interests. To reasonably appreciate the biological phenomena associated with these interests, the students must not only acquire principles and pertinent data, but they must also have effective training and assistance in the intelligent use of these data. In both these respects the students find us unprepared with sufficient suitable literature.

As an instructor in general botany who must daily face this deficiency in literature, I want to voice a plea to those who are interested in supplying content in printed form to make a more thorough survey both of the needs of the student and of the most suitable data and inferences now available in scientific literature—to approach this task as seriously and as open-mindedly as they approach research intended to advance the science of botany. In spite of our waning interests in the announcement of one new text book after another, I am going to advocate not fewer books but more of them—but they are to be unlike the common run of text books. With my topic thus limited I can soon be more specific.

Content should be subservient to and dependent upon objectives.

There seems to be an agreement as to the general objectives of our courses, but we have some differences of opinion as to what constitutes the attainment of these objectives and as to what are the most effective methods of attaining them. The study of plant materials at first hand in the laboratory and field is a common commendable practice. Some instructors require the student to make observations with little or no aid and spend considerable time in recording data largely in the form of drawings or diagrams. Later, when these records are checked, the student is informed whether his observations were correct. Others regard the presence of materials as furnishing the most appropriate setting for discussing the accuracy of observations being made, and also the significance of the facts observed. Daily class periods of the latter become discussions in the presence of materials. The traditional type of laboratory manual is inadequate for this type of course. Something more comprehensive and analytical is needed; call it a work book or what you will. It should contain a number of different features, such as suggestions and questions regarding observations to be made; aid to the recording of observed data (diagrams, graphs, blank charts, etc.); tabulation of additional data needed as a setting for certain learning situations; problems calling for further use of data in new situations; plans for summaries; problems and questions leading to a recall and weaving together of what has been accomplished concerning various topics from time to time. The whole should be so planned and organized that the student may readily be aware of his continuous development. Such an organization requires a careful balancing of time and types of learning situations in relation to the students' ability to comprehend and apply each major principle, and also further planning for its frequent recall and further application as the course advances. At Ohio State we are making our first attempt with this type of work book[1] in the hands of the student, in lieu of the old type manual supplemented by numerous mimeographed pages. We have yet to make a single departure that someone elsewhere has not also had under consideration. Hence it is with sympathetic interest rather than surprise that I find Professor Dietz preparing a similar work book for the general course here at Iowa State.

After the student has become interested in a project thus approached by observation, accompanied by discussion, and has fairly exhausted the facilities provided for him, he may want to extend his knowledge and ideas by reading. To supply this want on the part of the student we need a text book and a variety of other books written directly to the students at this level of development. This statement needs amplification.

One of the best examples that has come to my attention of a text book on a biological subject written directly to the student is the manuscript of a text book on The Principles of Heredity by L. H. Snyder. This author makes abundant use of the experiences of the students as points of departure, keeps the students clearly aware of progress made, and anticipates with them what is in store for the future. The whole procedure is replete with personal interests and challenges. The author avoids telling all he knows about a topic the first time it is introduced, or even all that he expects the students to know about it before the course is completed. What

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[1] Lithographed by Edwards Brothers, Ann Arbor, Michigan.

the student is capable of comprehending and applying is sufficient for the moment; then the next easiest, related, and already anticipated problem is encountered. Previous facts and principles are recalled when needed, the new facts met are all pertinent and needed at the moment of introduction. The book stands in sharp contrast to texts written with the sole objective of describing the facts of the subject, however valuable such text books may be.

Text books of general botany following this plan of procedure will help us to make more progress than we are now making. It is unfair to claim that authors of general botany text books have not made some attempt to write to the student, but it is fair to claim that the goal reached is inferior to the one they are capable of reaching.

But such a text book in general botany, while most desirable, is not enough. The students have a variety of interests. One group of students becomes intensely interested in one idea or project, another group in still another project, and so on. Each group asks for more literature on these special subjects. It may be the anatomy of leaves, a special group of plants, photosynthesis, the ecology of lawns, mutation, or any one of a number of other topics. To meet this desire on the part of the student before it is lost, we should have a number of small books or monographs that are appropriate for collateral reading on such topics—books in which the author develops a limited number of specific ideas and shows their relation to thought and practice. With our large enrollments such books would have to be supplied in quantity in departmental libraries, or by student rental libraries.

Perhaps the authors of such books may also include the average intelligent citizen in their reading audience, but they should strictly avoid all urges to contribute to specialtists in the subject. *The Living Cycads,* by C. J. Chamberlain, and *How We Inherit,* by Edgar Altenburg, are examples that closely approach what is needed.

The level of development of the reading audience should be carefully scrutinized by the author. A noted humorist once remarked, "It is not my ignorance that done me up; it was knowing too many things that aren't true." This statement aply describes one of the common handicaps of students in the general botany courses. Another of their handicaps, equally great, is their lack of both inclination and skill in the use of data as a basis of arriving at conclusions about biological phenomena. No author who wishes to write effectively to the general botany student can afford to blandly ignore these handicaps. Neither can he afford to assume that the students, unaided, will see what he may regard as the obvious relations and applications of the phenomena being described. The significance and application of data should not be taken for granted. The practice of obscuring facts and ideas with ill-advised as well as unnecessary technical terminology is still too prevalent in botanical literature for the most effective writing to the uninitiated. Charles E. Bessey and John M. Coulter still receive credit for their contributions to the evaluation and simplification of the botanical terminology of their time. We ought to profit by their example. For instance, a prominent specialist of the green algae maintains that he could give a complete scientific description of this group of plants, including a key, by using only five of the two dozen or

more names of spores current in the literature of this group.

There is time for a brief consideration of one more idea about the content of literature for general botany students. Only a few of the students in the general course are looking forward to botany as a profession. For the vast majority, the course is of value only as it contributes to a liberal education and functions as a general service course. Perhaps all thoughtful persons will agree that the scientific outlook on life does not encompass the whole of our interests in life. But one of our special assignments among the departments of a college is to furnish, through the medium of botany, those opportunities by which students may acquire a scientific attitude and the habit of using the scientific method when confronted with biological phenomena.

It is here that we meet one of our greatest deficiencies in the supply of suitable reading material for the general student. So much of our botanical literature is written in non-scientific and even anti-scientific language; i. e. language that is not consistent with discoveries in the field of botany as a whole—even language that sometimes renders superfluous all the data and inferences of some subdivision of botany, such as physiology or genetics. Rickett and other members of this audience have already called attention to this deficiency in published articles. But the deficiency is so prevalent and such a weighty handicap to progress that we should keep pounding away until the indolent become more circumspect.

While one might spend hours citing examples of this deficiency in published statements relating to diffusion phenomena, energy transformation, nutrition, culture solutions, ecological anatomy and other types of fluctuations, adaptation, natural selection, bases of interpretation, and a number of other phenomena; let us choose for the present moment the one encountered in every biological phenomenon discussed and one which seems to be the greatest handicap to clear writing in both description and exposition; namely, bases of interpretation.

One may wish to agree with the arguments of Kepner published in Science of July 6; and thus by personifying plants, he may then describe and explain their processes, activities and structures on the basis of purposeful teleology. If so, the least one could ask of him is that he be consistent enough to include all types of structures and activities however useless or destructive; that he discover whether the plant accomplishes the purposes he ascribes to it or whether it frequently makes mistakes; and finally that he frankly admit to the unsuspecting student that he is omitting data and analyses of data that other botanists deem necessary to explain these same phenomena on the basis of dependent relations—an interpretation in which each phenomenon observed is regarded as the result of preceding events with no planning whatever on the part of the plant. Such a book frankly written could be referred to for the point of view it exemplifies.

The worst books in this respect are those in which the authors indolently switch from one basis of interpretation to the other, depending upon their interest in and knowledge of the phenomena being discussed; or those books in which the authors employ the careless habit of stating facts in language that implies an interpretation which they themselves will not accept when critically questioned. Carelessness in expression is one

of the greatest handicaps that an author or instructor can put before a student beginning the study of a new subject.

We need more literature for the general student that describes dependent relations in language that does not violate the point of view generally referred to as that of cause and result—or, if you prefer—the point of view that certain sequences of phenomena are the consequences of their dependent relations. This point of view has the value of helping the student discover the limits of his present abilities and perceive further problems to be solved. The content and organization of a text book written from this point of view must of course be very different from that of a book written from the point of view of personification, since the author of the latter may omit data and exposition necessary to demonstrate that certain sequences of phenomena are consequences of their dependent relations. The instructor in the classroom should, of course, feel obligated to see that the students have ample opportunity to weigh both points of view for those phenomena with which they have familiarity and about which ample data are available.

To sum up: in every generation changing conditions result in new problems and a shifting of emphasis in regard to old problems. As a group, botanists now are facing annually tens of thousands of students in the general course. To serve them best, it is becoming more and more necessary to regard the general course as somewhat unique when compared with the more advanced courses for special groups of students, and therefore as requiring special consideration not fully accorded to it in the past. There should be a shift from the point of view of teaching subject matter to the point of view of teaching students through the medium of subject matter. On the basis of my experiences and those of my colleagues, I believe that an alteration in the types of books written for the general student, accompanied by a greater consideration for clearer and more effective writing, is one of the urgent problems of the present generation. In this short paper I have tried to show that we would be greatly helped by a combination of books consisting of (1) a work book to be used in an approach to plant science through first hand observations, (2) a text book written directly to the students to help them correlate fundamental facts, principles and applications in a manner that functions, and (3) smaller books for collateral reading to meet the demands of special interests. We shall have to meet such problems with courage and hope of accomplishment or be content to see botany become a profession of interest to specialists only.

THE DEVELOPMENT OF THE GROUP-CONFERENCE SYSTEM OF TEACHING

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When Charles Edwin Bessey introduced the laboratory method into botanical teaching the change was largely from the abstract to the concrete. This splendid achievement in teaching was very soon incorporated in the curricula of nearly all schools of higher learning and to some extent into the better high schools.

In the early stages of laboratory teaching the student number was small and the professor was able to give a great deal of personal attention to the laboratory work. However, when the student number increased sharply as it did at the beginning of the 20th century, the professor's time was fully occupied with the lectures and the laboratory teaching had to be released to an inexperienced teacher. This practice often resulted in so little contact between the lecture and laboratory that sometimes a lack of continuity and undue duplication occurred. Further, in the lecture-laboratory system of teaching the main objective was the imparting of information. Important as this may be, it has been recognized during the past decade that general botany must be more than subjective if it is to contribute its share to education. As early as 1911, at a conference on teaching held in Minneapolis, Dr. O. W. Caldwell showed a clear understanding of the problem when he stated, "If we can devise methods of making a scientific study of botanical education, we can improve our student product." However, this challenge has gone unheeded and no quantitative data have been made available. It is not within the scope of this paper to review the experimental work that has been done in other fields of science and at the different levels.

In 1926 the general botany staff of Iowa State College made their first concerted effort to improve the teaching of botany by applying the scientific method to their problem. The group-conference system is a product of this effort and has been gradually evolved out of the experimental results obtained. It is not looked upon as a finished product, but enough progress has been made to definitely differentiate this method of teaching from the usual laboratory-recitation-lecture system. The group-conference system makes the subject matter more concrete and makes it possible for the student to think in the subject using information and results accumulated individually and in groups. It permits making general botany more than subjective by placing emphasis on the use of information in thinking in the subject. The chief function of the teacher is that of a consultant for the students in their observations, experiments and interpretations, through suggestions, questions and directions.

From the experimental standpoint this paper will deal with the time and distribution of student contacts, on efficiency in general botany teaching, and with the use of the examination as a classroom tool in helping the student to think in the subject and finally with a discussion of some aspects of the group-conference system.

DISTRIBUTION OF RECITATION AND LABORATORY TIME

In 1926 the general botany courses were organized on a lecture-laboratory basis with no definite data available indicating the proper distribution of time between the lecture and laboratory. Data on these points for three representative years will be presented. In 1930, eight sections of general botany had the following distribution of time:

Number of recitations	Time in	Number of sections
1	3-2 hrs.	1
3	1-3 hrs.	3
0	3-2 hrs.	3
2	3-2 hrs.	1

All of these sections would be classified as small, ranging from 12 to 29 students. Each section was in charge of a well trained, experienced teacher, and one graduate student. Each teacher was given considerable leeway in the method of presentation, but the same subject matter was used in all sections. It should be noted that previous to this time the formal lecture had been eliminated from general botany.

The average grade received in a uniform final examination given at the end of the course was used as a criterion of the efficiency of distribution of time. The questions of this uniform examination were written by a committee of the instructors in charge of the general courses, none of whom met any student after the questions were selected. The personal element was eliminated by assigning each student a number as a means of identification. Further, all of the answers to each respective question were graded by one person.

A summary of the results showed that the three sections having no definite recitation averaged the same, namely 69, as the three sections having three recitations and one three-hour laboratory period. The advisability of having recitations separate from the laboratory was somewhat doubtful.

LENGTH OF LABORATORY PERIOD

The most efficient length of laboratory period was studied during 1931. The data are presented in table 1. The preliminary ratings of the students were computed by the Vocational Education department combining the aptitude test scores (weighted) with the average grade in high school mathematics. In case of agricultural and engineering freshmen the coefficient so computed had a positive correlation of .60; and when the high school averages were combined with the aptitude test scores (weighted), the correlation of the combined scores with college averages in the case of freshmen, industrial science students, was .59; while in the case of home economics students it was .67. The correlation between the preliminary ratings and the student's ability in botany as expressed

TABLE 1. *The influence of length of laboratory period on the grades received in the uniform general botany examination given December, 1931*

Section	Hours per week				Record final examination		Preliminary rating	
	Rec.	Laboratory		Total hrs. in lab.	Average	Rank	Average	Rank
		No.	Hrs.					
130X	2	2	3	6	59.75	1	2.88	5
130Y	2	2	3	6	48.40	4	2.77	1–2
130Z	2	2	3	6	48.26	5	3.09	6
130	2	2	3	6	51.32	2	2.91	2
135A	0	3	2	6	51.50	3	2.81	3
135B	0	3	2	6	54.30	2	2.85	4
135C	0	3	2	6	48.15	6	2.77	1–2
135	0	3	2	6	51.35	1	2.80	1

by the final grade in the uniform examination given at the end of the course in 1931 was .43. Although this coefficient was significant, a better criterion should be sought. In these studies the group of students was divided on the basis of their preliminary rating scores into five subgroups: The first subgroup or those rating "1" constituted the highest five per cent, the "2's" the next 20 per cent, and the "3's" the middle 50 per cent, the "4's" the next 20 per cent, and the "5's" the lowest five per cent (table 1).

It will be noted that the three sections having two recitations and two-three hour laboratories per week averaged about the same as the three sections having no formal recitations and three-two hour conferences per week, although the average preliminary rating of the former was .11 lower than the latter. Although the group-conference system as now practiced had not yet been used, it should be noted that there is really a comparison between the lecture-laboratory and group-conference systems presented in table 1 in addition to comparative results on the duration of laboratory. The results through 1931 suggested eliminating the formal lectures and recitation and shortening the laboratory period to two-hours. The most efficient number of contacts per week was still an open question.

STUDENT CONTACTS PER WEEK

In 1932 the student contacts per week was studied. The results obtained are recorded in table 2 and show a comparison of four sections having three, two-hour contacts with two sections having four, two-hour contacts. In this case the preliminary rating of the former group averaged .14 lower than the latter. On the basis of a uniform final examination given to all six sections there seemed to be little advantage in four contacts per week.

These experiments suggested the group-conference of three, two-hour contacts per week. Other questions such as the place of botany in the curricula and the desirability of preliminary sectioning of students according to their ability will be reported later in this program.

TABLE 2. *The influence of number of contact periods on the grades received in the uniform general botany examination, given December, 1932*

Sec-tion	Hours per week			Record final examination		Preliminary ratings	
	Number	Hours	Total	Average	Rank	Average	Rank
129	3	2	6	70.40	1	3.33	4
130A	3	2	6	58.27	5	2.86	1
135B	3	2	6	57.30	6	3.40	5
135C	3	2	6	61.88	3	3.50	6
				60.09	2	3.28	2
130X	4	2	8	58.43	4	3.10	2
130Z	4	2	8	63.33	2	3.20	3
				60.30	1	3.14	1

THE USE OF THE EXAMINATION

The examination has long been used as a measure of the students' ability in a specific subject and more recently as a measure of students' progress towards an education, the latter having a much broader scope. Little has been done, however, in using the examination as a means of teaching rather than a measure of ability or progress. In the following experiments an attempt is made to evaluate the examination as a means of teaching.

The exerimentation in 1931 involved 132 students in eight sections and that in 1932, 127 students in seven sections. All 15 sections would be considered small, averaging around 20 students. Not all the students in each section were included as the preliminary ratings were available for only 259 students.

The questions of the uniform examinations (Appendix A and B) were divided on the basis of "thought", "information" and mixed ("thought" and "information") questions. Only the first two will be considered in this paper. The "thought" questions were those which called for the solving of a new situation by using the information gained in class merely as a tool in the solution, while "information" questions were those which tested the retention of facts learned.

The uniform final examinations consisted of 10 questions, compiled and corrected as described earlier. The preliminary rating was also described earlier. The class tests were divided into announced and unannounced tests and the questions divided into "thought" and "information". The announced tests were those which perhaps tested the true ability of the students to cope with a particular situation. These tests were usually ten minutes in length. After the papers were taken up, a discussion and solution of the question followed. The papers were graded and returned at the next class contact.

The time spent on each kind of question was determined and an arbitrary coefficient was obtained by dividing the total minutes spent in writing tests by the number of hours of classroom contact of each

section. Obviously the higher the coefficient, the greater the amount of time spent in examination.

The average grade per section on each kind of question in the uniform examination was compared with the coefficient for time devoted to that kind of question in class. This was the basis for determining the relative value of each kind of question in developing ability to answer questions.

THE EFFECT OF "THOUGHT" QUESTIONS ON THE STUDENT'S ABILITY IN BOTANY

The different sections showed considerable variation in their ability to answer the "thought" questions (Appendix A) in the uniform examination given in December, 1931 (table 3). A perfect score on one question was 10. Section 136 ranked uniformly high on all of the "thought" questions, while 130Z and 135B ranked uniformly low on all of the "thought" questions. Within the sections there was considerable variation in ability to answer the various "thought" questions. Section 129 ranked first on question 10 and sixth on question 9, while section 130X ranged from first on question 9 to eighth on question 4. Section 136 with the lowest preliminary rating, but with a high coefficient for "thought" questions in class, ranked first on "thought" questions. Probably the low ranking for section 135B, in spite of its high coefficient for "thought" questions, could be accounted for by the fact that the "thought" questions were questions in announced rather than in unannounced tests as was the case in 136. Section 129, having the highest preliminary rating and a low coefficient for "thought" questions ranked second in ability to answer "thought" questions, and sections 130Y and 135C, having high preliminary ratings and low coefficients for "thought" questions ranked comparatively high (fourth and third) on "thought" questions. It seemed that the sections with high preliminary ratings were able to answer "thought" questions without preliminary training in class.

Similar results were obtained on questions 2, 4, 6 and 8 in the uniform examination given December 1932 (table 4). Section 130Z ranked uniformly high on all of the "thought" questions, while sections 130Y and 135A ranked uniformly low on all "thought" questions.

Much variation in ability to answer the various "thought" questions was shown in section 129 which ranked first on question one, but only sixth on question six; section 130 and 136 ranked first on questions four and eight and sixth on question two.

In table 4, section 129, ranking sixth on preliminary rating but with a high coefficient for "thought" questions in class, ranked second on "thought" questions. Sections 135A and 130Y, ranking high on preliminary ratings, ranked seventh and sixth respectively on "thought" questions.

THE USE OF "THOUGHT" QUESTIONS IN TEACHING GENERAL BOTANY

Although it appeared that the use of "thought" questions was an efficient method for developing ability in botany, there still remained the factor of the method of giving the "thought" questions in class.

Announced and unannounced tests were commonly used. An attempt was made to find the relationship between the student's ability, as expressed by his average grade on "thought" questions in the uniform examination, and the amount of time devoted to "thought" questions

TABLE 3. *The averages and rankings of sections on "thought" questions in the uniform general botany examinations given December, 1931*

Section number	Number of students	Average grade and rank on question, numbers:				Total grade and rank on all questions		Time devoted to questions in class		Preliminary rating	
		1	4	9	10	Average	Rank	Coefficient	Rank	Average	Rank
129	15	6.16[2]*	7.46[2]	2.93[8]	7.36[1]	23.91	2	.22	7	2.73	1
130X	16	5.91[3]	5.31[8]	3.87[1]	5.62[9]	20.71	5	.73	3	2.88	6
130Y	22	4.0[7]	8.27[2]	3.81[3]	5.13[8]	21.21	4	.31	5	2.77	2-3
130Z	23	4.0[7]	6.65[6]	2.65[7]	6.43[5]	19.73	7	.31	6	3.09	7
135A	16	4.75[4]	6.75[5]	2.06[8]	6.81[2]	20.37	6	.59	4	2.81	4
135B	13	4.46[5]	6.53[7]	3.15[5]	5.38[7]	19.52	8	.92	1	2.85	5
135C	13	3.61[6]	7.39[3]	3.84[2]	6.53[4]	21.37	3	.10	8	2.77	2-3
136	14	7.14[1]	8.28[1]	3.64[4]	6.58[3]	25.64	1	.83	2	3.36	8
Ave. for all sections		4.91	7.09	3.22	6.18	21.40					

* Upper number signifies rank on question; lower number signifies average grade on question.

TABLE 4. *The average and rankings of sections on "thought" questions in the uniform general botany examinations given December, 1932*

Section number	Number of students	Average grade and rank on question, numbers:				Total grade and rank on all questions		Time devoted to questions in class		Preliminary rating	
		2	4	6	8	Average	Rank	Coefficient	Rank	Average	Rank
129	15	7.47¹*	6.20³	6.73⁶	6.13⁵	26.53	2	.88	2	3.33	5
130Y	21	6.19⁷	5.52⁴	6.33⁷	4.05⁶	22.09	6	.36	3–4	3.10	3
130Z	15	7.00²⁻³	5.53⁵	7.67²	4.87⁴	25.07	4	.36	3–4	3.20	4
130 & 136	25	6.40⁶	6.40¹	7.64³	6.84¹	27.28	1	.94	1	3.08	2
135A	15	6.67⁴	4.80⁷	7.00⁵	3.26⁷	21.73	7	.28	5	2.86	1
135B	20	7.00²⁻³	5.15⁶	7.45⁴	4.60⁵	24.20	5	0.00	7	3.40	6
135C	16	6.44⁵	5.50²	8.25¹	5.19³	25.38	3	.14	6	3.50	7
Ave. for all sections		6.69	5.63	7.29	5.20	24.81					

* Upper number signifies rank on question; lower signifies average grade on question.

throughout the term, as expressed by the coefficient for time devoted to "thought" questions.

In table 5, sections 130X and 136, having low preliminary ratings and high coefficients for unannounced tests in class, ranked high on "thought" questions. Sections 129 and 135C, with high preliminary ratings and high coefficients for "thought" questions, also ranked high on "thought" questions.

Sections 135B and 130Z, with low preliminary ratings and low coefficients for unannounced "thought" questions in class, ranked seventh and eighth on "thought" questions in the uniform examination.

The results in table 6 corroborated the results of table 5. Section 129, with a low preliminary rating and a high coefficient for unannounced questions in class, ranked second on "thought" questions in the uniform examination. Sections 130 and 136 and 130Z, with high preliminary ratings and high coefficients for unannounced questions in class, ranked

TABLE 5. *A comparison of the average grades on "thought" questions in the uniform final examination with the coefficients for "thought" questions used in teaching general botany during the fall quarter, 1931*

Section number	Number of students	Total grade on "thought" questions		Coefficients for questions used in class		Preliminary rating	
		Average	Rank	An- nounced	Unan- nounced	Average	Rank
129	15	23.91	2	0.18	0.04	2.73	1
130X	16	20.71	5	0.0	0.83	2.88	6
130Y	22	21.21	4	0.31	0.0	2.77	2–3
130Z	23	19.73	7	0.31	0.0	3.09	7
135A	16	20.37	6	0.59	0.0	2.81	4
135B	13	19.52	8	0.93	0.0	2.85	5
135C	13	21.37	3	0.0	0.20	2.77	2–3
136	14	25.64	1	0.0	0.67	3.36	8

first and fourth on the "thought" questions in the uniform examination. Section 135B, with a low preliminary rating and a low coefficient for unannounced "thought" questions, ranked low on the "thought" questions of the uniform final examination.

Tables 5 and 6, for 1931 and 1932 respectively, showed that there was a pronounced tendency for the sections having high coefficients for unannounced "thought" questions in class to rank high in ability to solve "thought" questions in the uniform final examination.

THE EFFECT OF "INFORMATION" QUESTIONS ON THE STUDENTS' ABILITY IN BOTANY

The questions considered "information" questions in the uniform final examination given in December, 1931, (Appendix A) were numbers 2, 3, 5 and 8. If "information" questions were considered alone, the results of which were tabulated in tables 7 and 8, there was a tendency for students' ability on "information" questions to be influenced by the time spent on "information" questions in class.

TABLE 6. *A comparison of the average grades on "thought" questions in the uniform general botany examination with the coefficients for "thought" questions used in teaching general botany during the fall quarter, 1932*

Section number	Number of students	Total grade on "thought" questions		Time devoted to "thought" questions in class Unannounced		Preliminary rating	
		Average	Rank	Coefficient	Rank	Average	Rank
129	15	26.53	2	0.88	2	3.33	5
130Y	21	22.09	6	0.36	3–4	3.10	3
130Z	15	25.07	4	0.36	3–4	3.20	4
130 & 136	25	27.28	1	0.94	1	3.08	2
135A	15	21.73	7	0.28	5	2.86	1
135B	20	24.20	5	0.0	7	3.40	6
135C	16	25.38	3	0.14	6	3.50	7

Sections 129 and 130X (table 7), having high coefficients for "information" questions used in class, showed marked ability in answering the "information" questions in the final examination, while sections 135C and 130Z, both having low coefficients for "information" questions in class, also ranked very low in ability to answer "information" questions in the uniform examination.

Section 129, having the highest preliminary rating and ranking first on coefficients for "information" questions in class, ranked second on ability to answer "information" questions, while sections 130Y and 135C, both with high preliminary rating and low coefficients for "information" questions in class, ranked seventh and eighth on the "information" questions in the uniform examination.

The six "information" questions in the uniform examination given in December, 1932, were numbers 1, 3, 5, 7, 9 and 10. Table 8 shows the comparative averages and rank of each section of general botany on each of the six "information" questions. Sections 129, 130 and 136, and 130Z, with high coefficients for "information" questions used in class, showed marked ability in answering the "information" questions in the uniform examination, ranking first, second and third, respectively; while sections 135A and 135B, with no "information" questions in class, ranked sixth and seventh on "information" questions in the uniform examination.

Section 130 and 136 ranked second on preliminary rating, first on coefficient for "information" questions used in class and second on ability to answer "information" questions in the uniform examination, while section 135A, ranking first on preliminary rating and having no "information" questions used in class, ranked sixth on "information" questions used in class, ranked sixth on "information" questions. Sections with high natural ability seemed to be aided by the use of "information" questions in class.

THE USE OF "INFORMATION" QUESTIONS

Although there seemed to be a definite relationship between the "information" questions used in class and the ability of the students to cope with "information" questions in the uniform final examination, there remained the problem of the efficiency of the methods of presentation.

TABLE 7. *The averages and rankings of sections on "information" questions in the uniform general botany examination given December, 1931*

Section number	Number of students	Average grade and rank on question, numbers:				Total grade and rank on all questions		Time devoted to questions in class		Preliminary rating	
		2	3	5	8	Average	Rank	Coefficient	Rank	Average	Rank
129	15	7.53^{4}*	5.13^{3}	7.60^{2}	4.40^{2}	24.66	2	1.26	1	2.73	1
130X	16	6.93^{3}	5.50^{2}	7.62^{1}	4.37^{3}	24.42	3	1.25	2	2.88	6
130Y	22	6.36^{6}	3.54^{6}	4.90^{5}	3.54^{6}	18.34	7	0.93	5–6	2.77	2–3
130Z	23	6.87^{4}	3.87^{5}	4.26^{7}	3.82^{5}	18.82	6	0.93	5–6	3.09	7
135A	16	5.87^{7}	3.50^{8}	6.12^{4}	4.31^{4}	19.80	5	1.14	4	2.81	4
135B	13	6.07^{6}	4.15^{4}	4.46^{6}	5.61^{1}	20.29	4	1.15	3	2.85	5
135C	13	5.20^{8}	3.53^{7}	4.00^{8}	3.77^{7}	16.50	8	0.41	8	2.77	2–3
136	14	8.71^{1}	5.57^{1}	7.50^{3}	3.92^{5}	25.70	1	0.83	7	3.36	8
Ave. for all sections		6.70	4.28	5.63	4.13	20.74					

* Upper number signifies rank on question; lower number signifies average grade on question.

TABLE 8. The averages and rankings of sections on "information" questions in the uniform general botany examination given December, 1932

Section number	Number students	Average grade and rank on question, numbers:						Total grade and rank on all questions		Time devoted to questions during term		Preliminary rating	
		1	3	5	7	9	10	Average	Rank	Coefficient	Rank	Average	Rank
129	15	7.06^1*	8.13^1	5.67^1	7.67^2	7.53^2	7.73^2	43.79	1	0.65	2	3.33	5
130Y	21	5.86^1	6.81^1	3.90^5	5.90^5	6.43^6	7.43^4	36.33	5	0.16	3–4	3.10	3
130Z	15	5.73^6	7.00^3	4.93^{3-4}	6.93^3	6.93^5	6.70^7	38.32	3	0.16	3–4	3.20	4
130 &													
136	25	6.20^3	7.16^2	5.32^2	8.44^1	7.92^1	7.44^3	42.48	2	0.68	1	3.08	2
135A	15	5.53^7	4.93^7	3.93^6	6.26^4	7.00^4	8.26^1	35.91	6	0.0	6–7	2.86	1
135B	20	5.75^5	5.40^6	4.40^5	4.80^7	5.60^7	7.20^6	33.15	7	0.0	6–7	3.40	6
135C	16	6.31^2	5.69^3	4.93^{3-4}	5.06^6	7.06^3	7.38^5	36.43	4	0.07	5	3.50	7
Ave. all sections		6.06	6.47	4.72	6.50	6.93	7.45	38.13				3.20	

* Upper number signifies rank on question; lower number signifies average grade on question.

There was a tendency for those sections with high coefficients for un-announced "information" questions used in class (sections 129, 130X, and 136 in table 7) to have the highest average on the "information" questions in the uniform examination. Sections 130X and 130Y having low coeffi-cients for unannounced "information" questions and high coefficients for announced questions, ranked sixth and seventh on the "information" questions in the uniform examination. Section 135C, having a very low coefficient for unannounced "information" questions and no announced "information" questions, ranked eighth on "information" questions.

The comparison of the average grades received on the uniform exami-nation with the coefficients for "information" questions in the uniform examination given in December, 1932, showed results similar to those obtained in 1931. (table 8). Sections 129, 130 and 136, and 130Z, with high coefficients for unannounced "information" questions in tests used in class, had high average grades on the "information" questions in the uniform examination. Sections 135A and 135B, with no "information" questions used in class, ranked sixth and seventh on the uniform exami-nation.

A COMPARISON OF THE EFFECT OF DIFFERENT TYPES OF QUESTIONS ON THE STUDENTS' ABILITY IN BOTANY

Although considerable variation in the ability of different sections to answer the different types of questions was shown (tables 3 to 8), comparison of the rankings of the sections on grades in the uniform examination and coefficients for questions in class tended to emphasize the value of tests as a teaching method. There appeared to be a definite relationship between time devoted to tests in class and the ability of the students in the uniform examinations in 1931. In sections 129, 130X and 136, the ranking on grades in the different types of questions seemed to be rather closely correlated with the time coefficient devoted to either "thought" or "information" questions used in class.

It appeared that the average preliminary rating of a section did not necessarily signify the ability of the section in botany as measured by the different types of questions in the uniform examination. Sections 129, 130Y, 135C all had high preliminary ratings but varied greatly in ability to answer the different types of questions on the uniform exami-nation.

Section 129, having a high coefficient for "information" questions used in class, ranked high (second and third) on the different types of questions of the final examination, while sections 130Y and 135C, both having low coefficients for both "information" and "thought" questions used in class, ranked fourth and third on "thought" questions and sixth and eighth on "information" questions. It seemed that students with high natural ability could answer "thought" questions better than other types of questions without preliminary training in class.

Sections 136, 130X and 130Z, all with low preliminary ratings, varied directly in their ability to answer the different types of questions in the uniform examination as their coefficients for "thought" and "information" questions used in class.

In sections 136 and 130X, which ranked low on preliminary ratings (eighth and sixth) and high on the coefficients for "thought" questions used in class, it seems to have been possible to develop ability to answer "thought" questions since these sections ranked first and fourth on

"thought" questions in the uniform examination. Section 130Z, having low coefficients for both "thought" and "information" questions used in class, ranked sixth and seventh, respectively on the different types of questions in the uniform examination.

Comparison of the rankings on grades in the uniform examination given in December, 1932, with the coefficients for different types of questions showed results similar to those obtained in 1931. In sections 129, 130 and 136 and 130Z, the grades in the different types of questions seemed to be rather closely correlated with the time devoted to either "thought" or "information" questions used in class.

Section 135A, with a high preliminary rating, and sections 129 and 135B, with low preliminary ratings, varied in their ability to answer the different types of questions in the uniform examination as their coefficients for "thought" and "information" questions used in class. It seems to have been possible in section 129 to develop ability to answer "thought" and "information" questions by the use of "thought" and "information" questions in class. Section 135C ranked high on "thought" questions in both examinations, although the coefficients for both types of questions in class were very low. This can probably be accounted for by the fact that "thought" questions were used in teaching, although they were given as problems to be solved orally in class or to be written and handed in the next day. For that reason the time spent on them was not recorded, and these questions were not apparent in the coefficients for "thought" questions used in class.

THE PRETEST

It has been shown that the short announced and unannounced tests were effective means of teaching botany when based upon the final examination as a measure of ability in botany. It would seem desirable to ascertain whether these same types of tests would be effective if measured by student progress.

In order to throw some light on the foregoing problem a pretest was given in the fall of 1931 to eight sections of general botany consisting of 159 students. This pretest was in the form of a true-false examination involving information over the first problem—how plants make food—five weeks' work. At the end of this period the same examination was given. These results should afford a measure of student progress. The average progress per section is shown in table 9. The average preliminary rating and the coefficients for tests used in class were computed as described earlier. In three sections there were no class tests given previous to the post-test, in one section only announced tests, in three sections only unannounced tests, while in one section announced and unannounced tests were given. These tests were further classified according to the relative time spent on "thought" and "information" questions. It should be noted that a minus score could be secured on this examination hence the section having the lowest average score in the pre-test could show the most progress without necessarily having the highest score on the post-test.

The three sections having no class tests and ranking second, third, and seventh in preliminary rating, ranked seventh, eighth and fourth in improvement (table 9) and sixth, eighth and fifth respectively in average post-test score. Those sections having the highest coefficient for "thought" questions used in class tests also ranked high in student progress and

TABLE 9. *Comparison of the scores received in a pre-test and post-test, true-false examination given, fall, 1931, with tests given in class*

Section	Number of students	Pre-test		Post-test		Improvement		Coefficients for tests given in class				Preliminary rating	
								Announced		Unannounced			
		Rank	Average	Rank	Average	Rank	Total	Thought	Information	Thought	Information	Rank	Ave.
129	16	7	−15.0	3	47.0	3	61.87			.04	.14	1	2.73
130X	18	5	−6.88	2	51.55	2	63.77			.36	.73	6	2.88
130Y	22	3	1.45	6	39.81	7	38.36					2–3	2.77
130Z	27	6	−9.36	5	42.81	4	52.44					7	3.09
135A	23	8	−29.0	7	37.73	1	67.17	.59	.23		.14	4	2.81
135B	19	2	4.21	4	44.84	6	40.63	.59	.23			5	2.85
125C	19	4	.21	8	35.78	8	34.73			.36	.31	2–3	2.77
136	15	1	12.53	1	56.80	5	44.26					8	3.36

highest in average post-test score. Section 135A had an inexperienced instructor which may in part account for the lack of correlation between class tests and student progress. With the exception of the above section, all sections using class tests as a means of teaching, ranked higher than those sections which did not.

<div align="center">"SPOT-QUIZ"</div>

The microscope is a valuable tool in teaching botany. The materials used necessitate that considerable time be devoted to manipulative skill with the microscope. Having acquired this technique it would seem desirable to combine skill in the use of the microscope with the examination as a means of teaching. Such a procedure, which has been effective in the general botany laboratories at Iowa State College will be described.

The ocular of each microscope is equipped with a pointer by taking a horsehair of sufficient length to reach to the middle of the field when it is placed and held fast by means of balsam on the middle diaphragm of the ocular.

Before any students enter the class room a microscope is placed at each student's position and a slip of paper bearing a number and directions is placed at the base of each microscope. A slide is adjusted on each microscope with the end of the pointer at the particular material to be studied. The students are directed to adjust only the micrometer screw, thus leaving the same material in place during the period. Each student places numbers, from one to the maximum number of microscopes to be observed, in consecutive order on a blank paper. Then starting with the microscope before him, each student makes his observations and writes on the corresponding blank space, the answer. A uniform time of one-half minute is given. At the word "shift" each student moves to the next microscope. This procedure continues until each student returns to the microscope with which he started. It is advantageous to have at least as many microscopes set up as there are students in the section. If, for any reason, a microscope should get out of adjustment the instructor's attention is called to it. Each student works independently forming personal opinions from independent observations.

The papers are exchanged and corrected in class by the students. The assistant writes the number and correct answer on the blackboard as the instructor makes his observation on each microscope. This procedure informs the instructor whether the microscope remained in adjustment during the period of student observation. The students are given approximately 10 minutes to reexamine materials upon which they were in error.

An example of a "spot-quiz" as this examination is called by the students, follows:

Number	Direction	Answer
1	What is it?	Potato starch grains
2	Function?	Strengthening (collenchyma)
3	What is it?	Air bubble
4	Kind of section?	Longitudinal section of a vascular bundle of corn
5	What for?	Carrier of determiners of inherited characters (chromosomes)
6	What is it?	Pollen tetrad
7	etc.	etc.

The materials for each examination are usually confined to a particular problem except at the end of the quarters when this type of examination is used as a review.

In the group-conference system, where, as will be shown later, much emphasis is placed upon observation in the presence of plant materials as a means of formulating ideas, the above type of examination has been found effective in encouraging students to observe. It is a distinct aid to the students in acquiring the information used in the gaining of concepts.

After the student has become familiar with the use of the examination as a means of teaching rather than a measure of his information, he prefers the former. His grade depends more upon his ability to use the information he possesses than the amount of information. Most of the "thought" questions require use of information.

THE GROUP-CONFERENCE SYSTEM

In the group-conference system all the teaching is done in the laboratory, greenhouse or field in the presence of plant materials. Discussions are held whenever the class has carefully studied the available material and has formulated opinions backed by observations. There are three two-hour conferences per week per year in the general course which is given largely to a non-professional group with less than one per cent of the students majoring in botany.

Administrators of courses have given considerable attention to objectives. It is apparent that students also might have objectives when taking a course, although this has heretofore received little attention. Such objectives should find a place in a course as they tend to afford a natural interest.

Each member of the regular staff in botany who teaches one or more sections has one to several research projects. This affords student contact with a teacher imbued with the research spirit. Further, the general courses are taught by two cytologists, one morphologist, one mycologist, three pathologists and two physiologists, each of whom contributes from his specialty at the weekly conference of the general botany staff. The presentation of the material from a broader view point tends to create interest among the teachers and the students.

ORGANIZATION

The organization of the course is on a concept basis. Exercises were eliminated as these tend to break the course into static units which the students view as tasks to be done. The content of the first quarter's work comprises three fundamental concepts, all oriented around seed plants. These are: (1) How plants get their food, (2) how plants grow and differentiate, and (3) how plants reproduce. These biological concepts were chosen because of the fundamental rôle they play in life. They collectively constitute the theme of the course.

The conferences on photosynthesis usually last five weeks for satisfactory completion. For convenience the concept on photosynthesis is subdivided into: (a) The factors involved in the manufacture of food, (b) the food-making mechanism, (c) the food substances formed, (d) the utilization of foods, (e) the by-products. The conferences on growth and reproduction take three and four weeks, respectively.

The second quarter applies the same concepts used for the flowering plants. No time is spent on evolution or learning life cycles. Evolution as such receives no emphasis on the study of the different forms. At the end of the course, with all life cycles before them, the students usually grasp the concept of evolution with little aid. Another characteristic of the work in the second quarter is the emphasis placed on the phenomenon of parasitism. The approach is not from the standpoint of studying the life cycle of a few forms like *Albugo candida, Erysiphe graminis* and *Puccinia coronata,* but rather of determining how various parasites obtain their foods, that is, through haustoria, intercellular and intracellular mycelium and secretion of enzymes. Closely allied with the concept of food-getting are the different methods of entrance of the parasites in the host as through wounds and stomata. Lastly, each small group of students is given an opportunity to inoculate several different hosts with certain pathogenes.

The spring quarter is spent largely in the field . There are usually five class projects, as follows: (a) Identification of trees in winter condition, (b) how to know spring flowers, (c) Iowa ferns, (d) organization and conducting of a field trip, (e) plant propagation. The students are encouraged to suggest their own projects.

HABIT OF SCIENTIFIC THINKING AND CREATING INTEREST

An attempt will be made to show how the group-conference system may develop in the student the habit of scientific thinking and create a permanent interest in plant life.

The logical steps in scientific thinking are usually considered to be: (a) Recognition of the problem, (b) the venture of a guess as to its solution, (c) the testing of this hypothesis, (d) the modification of the hypothesis in light of the findings, (e) retesting until all evidence points to the attainment of a valid theory, (f) the stating of conclusions, theory, or law, (g) the application of the accepted conclusions, theory or law to situations solvable through its use.

Let us now consider how botany may be taught while developing the habit of scientific thinking. As stated earlier, the course is organized on a concept basis; these concepts are subdivided. The student is given experience in developing his ability to recognize the concepts; moreover he is encouraged to suggest additional and original sub-concepts. Since few or no laboratory directions are given, a clear-cut understanding of the problem is necessary for efficient solution. Early in the course, considerable effort is exerted by the instructor to make the student feel that the laboratory is his and that he may use it as a place in which he can work out his problems.

The acquisition of botanical information does not appear as a course objective. The viewpoint is developed among the students that the acquisition of information is merely a tool for the development of concepts and not the ultimate goal of the course. The information acquired by the students during the course is secured by encouraging wide biological reading, experimentation, topic assignment and the use of the book as a reference. Previous experiences also supply some of the information.

An attempt is made to so orient the field of botany around the individual student that his ability to recognize and formulate concepts will be augmented. This method is in sharp contrast with the more usual practice

of orienting the student around the field of botany. The technique is largely one of repeated use of information.

Experimentation is one of the fundamentals of science. Students will be and should be somewhat hesitant about doing experimentation spontaneously unless there is considerable doubt about what the results will be. This doubt encourages the venture of a guess as to the solution of the problem. To illustrate: suppose the students have learned by previous experimentation and discussion that cholorphyll, water, light and carbon dioxide are essential for photosynthesis. The class discussion has led to the question of how the carbon dioxide comes in contact with the chlorophyll. The student guesses may range from some sort of valves at the base of the petiole, to openings in the leaf. Experiments lead to the discovery of stomata and the application of the laws of diffusion.

To illustrate the rôle of experimentation in developing the scientific habit of thinking, a portion of the concept on photosynthesis—the factors involved—will be used. The syllabus gives the following suggestions: "Secure two similar geranium plants. Test a leaf of each for starch. Place one plant in total darkness for 48 hours and expose the other to continuous light for the same length of time. Remove several leaves from each plant and test for starch." As you know, this simple experiment shows the necessity of light for photosynthesis. In discussion, the question is raised whether the necessity for light is proven. The idea that proof requires repetition is always forthcoming. In addition, retrial with other plants is made and the effect of the leaf's position on the plant is discussed.

The syllabus makes the following suggestions: "Transfer a branch of Elodea which is bubbling vigorously to boiled and cooled carbon dioxide free water." As you know, this is a simple experiment to teach the necessity of carbon dioxide for photosynthesis by water plants. In performing this experiment the classes have always worked around to the point of substituting "number of bubbles given off per unit of time" for "bubbling vigorously," thereby establishing the idea of quantitative rather than qualitative measures in experimentation. Further, the classes have suggested additional experiments such as: blowing carbon dioxide in the boiled water, transferring the Elodea plant back to the original container, and the value of repetition.

Again the syllabus suggests: "A potted plant and an open dish of soda-lime are placed under a bell-jar on a glass plate. A seal of vaseline is made between the jar and the plate. Expose to normal light. Keep a check plant outside of the bell jar. After two days test both plants for starch." The students have usually substituted "continuous light of a known intensity" for "normal." They have modified the experiment so that both plants are under bell-jars, one being supplied with carbon dioxide by blowing in of the breath. This brings out the idea of varying only one factor at a time in experimentation.

It will be seen that not only have the students learned the facts that light and carbon dioxide are necessary for photosynthesis but they have gained experience in the following cardinal principles of experimentation: (a) Value of repetition, (b) value of quantitative measure, (c) value of varying only one factor at a time, (d) necessity of a control or check. The student has been allowed to take the initiative in suggesting supplementary problems in which he was not merely following directions and in addition has been given valuable training in critical thinking.

The application of the accepted conclusions, theory or law is largely accomplished by the use of five to ten minute examinations given at each class session in which the student is asked to apply the information he has gained in class. Example of thought questions are: (1) What is the minimum number of pollen grains required to produce a watermelon containing 350 seeds? (2) How does a white corn seedling gets its food? (3) The lower epidermis of the Wandering Jew strips much more easily than the upper. Explain. (4) Design an ideal food-making leaf.

These questions call for the application of a principle to a new situation, the interpretation and use of data or the introduction of a new situation. The object of the latter is to allow the students to think about the new situation so that it crystallized the information they may have gained through earlier experiences and makes them more receptive.

Lastly an attempt is made to meet the student-objectivs for the course. In a study over an eight-year period the student objectives were classified under 11 categories which ranked in preference as follows: How to know plants, increased knowledge, cultural, professional, economic importance, structure and function required, diseases, growth, environment and evolution. The fact that the student is allowed the initiative in choosing his own project promotes thinking in the subject.

PERMANENT INTEREST IN PLANT LIFE

The objective of developing in the students a permanent interest in plants is attempted through many techniques.

Experimentation is an unusually valuable tool in the group-conference system. However in the minds of most of the students experiments serve to prove something or verify some fact which they already know. An effort is made in the group-conference classes to create the impression that experiments may not necessarily prove anything, but are merely tools used in the search for the truth. Allowing the students considerable freedom in designing and performing their own experiments, they usually start with something familiar and are therefore not involved in a long series of static and sometimes uncomprehensive directions. One or two experiments are usually in progress during the total time spent on a concept. Interest is often created and maintained by the type of experimentation used.

The formal lectures have been eliminated. Group-conferences are held informally in the presence of materials. This allows a student to have all his contacts in botany with one instructor, thereby reducing duplication. The students are encouraged to take no notes during discussion, but to understand the principles and inferences made.

The terminology is reduced to the minimum necessary for understanding a concept. The students are allowed to feel the need for a term before it is introduced. For example, a trip to the greenhouse or field during which a study was being made of the relationship of the parts of a leaf to photosynthesis, the instructor might pick several types of leaves and show how the petiole was able to orient the blade in relation to light. The students would probably speak of the petiole as the "stem" of the leaf or ask what it was called. The term, "petiole," once introduced, would be used to aid in retention.

A course may usually be made more concrete and dynamic by devoting a major part of the student's time in thinking in the subject rather than

about it. The former requires considerable understanding while the latter may be the product of a good memory. Thinking in a subject is augmented by spending considerable time in using information rather than all the time in acquiring it. There is apparently a close correlation between understanding and interest.

The students are encouraged to read broadly in biological literature. The textbook is used mostly as a source of information for understanding a concept. A group of 30 books is kept on a special shelf to which students are allowed free access. Each student is required to select and review one book from this list per quarter. Such books as "Hunger Fighters," by DeKruif; "The Green Leaf," by McDougal; "Possible Worlds," by Haldane, and "Biology and Its Makers," by Locy, have been found effective.

An effort has been made to remove unnecessary routine to stimulate student interest. The difficult and time-consuming drawings, such as cross sections of stems, are furnished. The student, when studying a woody stem is a tool in the concept of plant growth, studies and interprets a large number of woody stems. He is then given a detailed drawing of one which he labels with the aid of a prepared slide. In the example mentioned, the time is spent on observation. The student is encouraged to make his own decisions on the relative importance of various portions of the concept. If he can portray an idea by a diagram or drawing, he is allowed to do so, but makes his own decision not only on the drawing but also the idea.

In the spring of the year the group-conference periods are mostly out-of-doors. The use of the out-of-doors as a laboratory has stimulated interest. The student is given a further opportunity to utilize the information accumulated during the first two quarters in thinking in the subject. The course content is organized around the expressed interest of the students. This reduces the necessity, on the part of the instructor, of striving for interest and enables him to devote more of his time directing, suggesting and questioning the student. The spirit of cooperation in meeting new situations and in thinking in the subject tends to increase student interest.

APPENDIX A—THE UNIFORM GENERAL BOTANY EXAMINATION GIVEN DECEMBER, 1931

1. Describe *in detail* the internal and external characteristics of an ideal, efficient, food manufacturing leaf (use diagrams if you wish).

2. A plant has 8 chromosomes in its leaf cells. How many are there in its pollen grains? Explain *in detail* how this comes about.

3. Describe 10 plant tissues and give the function of each.

4. Southern sweet potato growers sometimes remove a part of the tops "to throw the strength of the plant into the roots." What should you advise them to do? Why?

5. With the aid of diagrams, explain the complete process of fertilization—starting with the pollen mother cell in the anther and the megaspore mother cell in the ovary through the formation of the seed.

8. How does a plant take in water and minerals? Trace the tissues and organs through which nitrates dissolved in soil water must pass to reach the food-manufacturing cells of the leaf.

9. If the leaves of a tree are destroyed by insects how does it influence the growth of the tree? Why?

10. Of what value to man is the photosynthetic process?

APPENDIX B—THE UNIFORM GENERAL BOTANY EXAMINATION GIVEN DECEMBER, 1932

1.   Describe the process of food-making in plants.

2.   Why are parallel veined leaves in monocots the most efficient type for crowded conditions as illustrated by grain fields and meadows?

Some plants grow well in the shade of other plants. What characteristics of the leaf of such plants fit them for growth under shaded conditions?

3.   Illustrate by diagrams and name all of the structures of the typical cell, and the nuclear changes incident to mitosis (cell division).

4.   Account for the following:

(a) White and yellow seedlings of corn occasionally are seen in the corn field; they soon die unless they turn green; some of them become green when supplied with iron.

(b) Around cement plants where there is much dust in the air, plants are frequently injured.

(c) The possible advantage to an oak tree of the annual loss of leaves.

(d) When ice cream freezers are emptied around shrubbery, the leaves wilt and the shrubbery may die.

5.   What tissues are common to the root, stem and leaf? Give the functions of each.

6.   How many pollen grains are necessary for the fertilization of an ear of corn? Aproximately how many must be borne by the anthers of the plant to insure complete fertilization under ordinary conditions? How has adaptation in some plants to insect pollination modified the amount and structure of the pollen and the structure of the stigma?

7.   In a well labeled diagram show he structure of a leaf as it appears when sections are examined.

8.   Explain the presence of red 'grains" of corn on a yellow ear. Why does not a budded Grimes Golden (yellow) apple tree bear some red apples when grown next to a Janothan (red) apple tree?

9.   Describe how plants get water and minerals from the soil.

10. True and false questions: (Mark with X in square)

True   False

(a) The yellow portion of the spectrum is most effective in photosynthesis

(b) Secondary roots are the absorptive organs of the plant

(c) The pollen mother cell has X number of chromosomes

(d) Tap roots hold shifting sands better than fibrous roots

(e) In dry areas fibrous roots are more successful than tap roots

(f) In photosynthesis, oxygen is taken in and water vapor given off

ATTAINMENTS IN TEACHING

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My main difficulty during this hour clearly lies in the fact that while educationists think they know what the attainments of good teaching are or really ought to be, not many other persons in higher institutions seem inclined to agree openly with them, though I suspect they do so in their own secret thoughts. However this be, we have long since learned not to be discouraged, to try, try again, not even to expect others to see things as we do and to keep sweet and sunny through it all.

People in our professional field sometimes say, "Good teaching begins where most teaching ends."

It is, of course, an unfortunate and tactless statement, of the general type for which educators have long been notorious. It really is meant not even to reflect upon, much less to condemn in a single, sweeping sentence, most teaching, but instead to arrest attention and to direct it to a critically important aspect of all really effective teaching.

It is this: That knowledge ought not to be regarded as an end in itself, but instead, merely as a means to other ends; and that whereas very much teaching ends with the acquisition of knowledge by the student, really good teaching regards this merely as a preliminary step and goes on to the far more difficult task of actually realizing the ends, or goals, which lie beyond, and to which the knowledge taught really leads.

The thought has been qutie well expressed by Dr. C. R. Mann, Director of the American Council on Education:

> "For 'knowledge' is not the purpose, objective or end of schooling. Sensible conduct or constructive action is. Knowledge is instrumental—a means. Therefore to treat knowledge as an end is to fall into one of the subtlest and most confusing of logical fallacies—to substitute the means for the end."

Into this subtle and dangerous fallacy of substituting the means for the end, very many teachers have doubtless fallen; and I suppose I might go on and say that the greatest of all attainments of teaching is following through to the ultimate goals that lie beyond the acquisition of knowledge —that is, making it actually function in the manner in which it must function if it is to have any value to the learner. However, this would be merely a vague and all but meaningless generalization. I shall try to be more specific.

Just what are the ends to which the knowledge that we teach to our pupils really should lead? For while we may see very clearly that this knowledge does not represent, in and for itself, the real end of the efforts of students and teachers, we may still find it difficult to say just what these ultimate ends really are. Neither has anyone found the final, unalterable answer. Of the identity of some, however, we may be quite certain.

INTERESTS AS ENDS TO BE SOUGHT BY TEACHERS

It is generally held, for example, that one of the most important of all outcomes of teaching—one of these ends to which knowledge is a means—is the development of strong, lasting interests in our pupils. Indeed, there are many who believe most sincerely that the persistent, enduring interests, which, sometimes at least, we develop in our young people, and which really determine what they will learn, what they will think about, what they will do, and even what they will become in the future are of greater actual worth to them than the knowledge which they laboriously acquire while under our instruction. Doubtless many of us, as we call up the memories of particular teachers, who in some way that we may not even yet quite understand generated such interests in us and started us on the roads that we have followed ever since, would agree that these interests are truly among the greatest of all attainments of teaching. At any rate, we should doubtless give them a place in the list of worthwhile outcomes of our work.

IDEALS AS OUTCOMES OF TEACHING

Another attainment of good teaching that seems worthy of the earnest efforts of teachers everywhere is the establishment of desirable ideals in their pupils. By an ideal I mean a high end or goal, which one desires so much to achieve that he is willing to put forth effort nearly without stint or limit in order to attain it. Such an end or goal may or may not be moral, or spiritual, in character. Thus one may pursue the ideal of living uprightly before his God and his fellow men, or just as truly, of acquiring an agreeable personality, or of achieving high distinction in some field of learning. Indeed, it is probably true that no person has ever traveled the long, hard road to high scholarship unless he was led by some high, strong ideal, for the attainment of which he was willing to pay the price. As has been said, we should apparently endeavor to develop deep, abiding interests in our students; but just as earnestly, we should doubtless strive to instill in them, by whatever means, ideals of high achievement, not unlike those by which we, ourselves, have been led through the years since first we accepted them as the remote and all but unattainable goals of our own efforts.

UNDERSTANDING AND ASSIMILATION AS AN END OF TEACHING EFFORT

I have spoken of two of the highest of all attainments of teaching. They relate to the development of the internal motives which alone can cause students to put forth the very best efforts of which they are capable. Lest I seem to have wandered out and away from the world of classroom realities, let me turn to things somewhat more orthodox in college teaching.

Most teachers endeavor to develop in their pupils what I shall call understandings. The word should be given a larger meaning than it usually carries. In this larger sense, understanding means comprehension or mental grasp, such as results only from a process of integration and true assimilation of knowledge. Thus a thing truly understood seems to have become a veritable part of the mind itself. We see students achieving such understandings occasionally, yet all too rarely. Always it warms our hearts; and always we are able to see clearly the vast difference between

knowledge so understood on the one hand, and knowledge merely memorized against a day of reckoning on the other. The student who grasps, who comprehends, who integrates, who assimilates, who truly understands his knowledge is the one for whom our own lives of service are really spent. In understandings, as I have used the term, we have, then, another of the transcendent attainments of good teaching.

A FOURTH GOAL THE ABILITY TO APPLY

Still another, and one quite different in character from the three already named, is the development of the actual ability to apply the knowledge acquired, particularly in situations of the kind in which it is to be met in later life. The easy thing in teaching, of course, is to require acquisition only, and to be satisfied when this is secured, regardless of whether the pupils have or have not developed the ability to make the applications of it which they must make in life beyond the school. Two facts seem self-evident: Mere acquisition of knowledge carries in itself little or no actual assurance of the ability to use it effectively in future years; and if our pupils do not become able to apply the knowledge which we teach, then there is no great use in having them acquire it at all. As Dr. Whitehead, one of our keenest contemporaries, has put it:

"You cannot become wise without some basis of knowledge; but you can easily acquire knowledge and remain bare of wisdom."

In the ability to apply or use knowledge effectively lies wisdom. It alone justifies acquisition. It must be regarded, therefore, as one of the critically important outcomes, or attainments, of all really good teaching.

THINKING ABILITY A MOST IMPORTANT OUTCOME

A fifth attainment of good teaching is the development in the pupils of the ability to think well.

Dr. Eliot, long president of Harvard, once said:

"From the first to the last, it is the teacher's most important function to make the pupil think accurately and express his thought with precision and force."

Speaking to the same point, Henry Ford has said:

"A man who cannot think is not an educated man, however many college degrees he may have acquired."

Of course, neither of these men had in mind only that sort of thinking which is done in courses in mathematics, where the processes and quality of thought receive most careful attention. They referred, just as truly, to the several other kinds of thinking that actually make up a far larger part of our mental life and play at least an equally important part in human affairs. Some teachers provide little or no opportunity for thinking of these kinds in their class work. Others are careful to do so. Of these, however, not many appear to require their classes actually to observe the rules

and standards of scientific thought, which should unquestionably be rigorously applied in all thinking of this sort.

At any rate it appears that the importance of this particular end, or goal, of teaching can hardly be overestimated. Among the greatest of modern educational tragedies is the failure, apparently through sheer neglect, to attain it.

RETENTION BY PUPILS AS AN ACHIEVEMENT OF GOOD TEACHING

Sixth in our list is an achievement of really good teachers, which may be regarded either as a by-product of those that have been named or as having been included in them, yet which is so important as to deserve a place in its own right. It is the retention by the students of the essential or fundamental knowledge which they have been required to learn throughout the period of their need for it. Too often we have been satisfied with mere acquisition of this knowledge and have left retention to take care of itself. Perhaps we have assumed that if the knowledge were acquired, it would be retained. Yet a moment's thought suffices to show the utter folly of any such assumption.

The importance of retention lies in the fact that it is one of the three limiting factors of thinking ability. An empty head cannot think for the very simple reason that factual knowledge is the raw material that enters into thinking. Put otherwise, no person's thinking is better than his information. It follows that an indispensable step in our effort to develop thinking ability in students is the furnishing of their minds with a store of knowledge of maximum usefulness, and this in such a manner that it will be retained against the time of need, whenever that may chance to be. Clearly, knowledge that is not retained cannot function in the manner in which it must if it is to have any value whatsoever in life.

THE ABILITY TO INFER AS A DESIRABLE OUTCOME

The list is growing long. Yet I think there are still two or three other highly important outcomes of good teaching that should be included in it.

One of these is the ability to draw sound inferences from known facts. We shall recognize this at once as a vital step in all scientific investigation. Few, however, have identified it as one of the most common processes performed by everybody in the daily round of life. Yet the truth is that every one of the innumerable decisions made by any intelligent human being in this modern world represents an inference, drawn from and based upon his knowledge and experience. It follows that the importance of right decisions in life is one with the importance of the ability to infer. Moreover, if our students are trained to do it, as they easily may be, they are quickly found extracting new meanings and seeing the hidden implications of the knowledge that they have acquired. Finally this ability, if developed, takes care automatically of very many of the so-called details with which our college courses tend to be crowded. Since the students can and do easily infer many such details, it apparently becomes unnecessary to teach them.

THE ABILITY TO FIND NEW KNOWLEDGE AN IMPORTANT END

It has been said by someone that to know where you can find a thing is the mark of an educated man.

Most of us doubtless would hold that this ability is at least one of the important marks of an educated person. Neither does this merely represent the obvious solution of our baffling problem of how to teach to young people bodies of knowledge surpassing by far the assimilative capacity of their finite minds. It does, of course, offer really effective help toward the solution of this troublesome problem. Yet from the standpoint of the students, the real value of the ability to find knowledge in a given field lies in the fact that a new world of thought has been made accessible to them. Given an understanding of a few of the basic principles of any subject, some familiarity with the literature of that field becomes necessary if we would leave open to them the door to further growth. Further than this, sound thinkers must be able to gather facts relevant to all sorts of problems arising in connection with the affairs of later life. Unless, then, we develop in our students the ability do do this in the various fields of knowledge, all our efforts to make them able to think well must prove more or less futile. Taken together, these considerations seem sufficient to place the ability to find knowledge when needed among the most important outcomes of effective teaching.

THE WHOLE END OF OUR EFFORTS

Among the transcendent ends of good teaching, I have named eight:

Abiding interests
Strong, worthy ideals
Broad, clear understandings
The ability to apply the knowledge acquired
The ability to think well
Retention of the essential knowledge
The ability to draw sound inferences
The ability to find new knowledge when it is needed.

Since these eight are really expressed in terms of student achievements which result from good teaching, it is interesting to inquire how nearly they constitute true scholarship. Is scholarship, in its finest sense, anything more than a composite of one's interests, ideals, understandings, abilities to apply knowledge, skill in thinking, knowledge retained, skill in drawing inferences and skill in finding new knowledge? Apparently they make up almost wholly the list of its component, essential elements. Or we may inquire instead whether, with these eight attainments known for any individual, we have the true measure of his education. Again it appears that they are the chief elements, the core and substance of an education in the very highest meaning of that term.

Finally, it may be fairly asked whether these ends can actually be attained, or whether instead they are simply outcomes that teachers may devoutly hope for but may never realize. The answer doubtless is that good teachers, of whom there are very many, seem actually to realize them with at least a large proportion of their students. This is, I think, a matter of common observation and experience. Of it, Edward Yeomans has written in this manner:

"This strange, subtle undercurrent, this wind of the spirit which bloweth where it listeth—which cannot be defined or confined or ex-

pressed in any formulae—this whole core and substance of the educational process can be passed. It can be passed on one condition and only one, namely, that the teacher is actually a source of illumination —not a reflected light, but a light producer; not a moon, but a sun; and that the scholar is capable of catching fire, is combustible, is spiritually organic."

CHANGING EMPHASIS IN GENERAL BOTANY AND ITS SIGNIFICANCE

C. F. HOTTES

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A meeting such as this augurs well. Botanists from widely different sections of our land, of diverse training and outlook, are gathered about the conference table to weigh the old in the light of the new. With an admirably conceived program before them, the participants, motivated by high ideals, enter into the discussions with the definiteness of a single purpose, namely, a better coordinated and more appealing introductory course in botany. I verily believe that what is here done will play no small part in remolding our instructional procedure, and, through it, revitalizing our science. If I mistake not, it will bring honor and credit to Iowa State College that numbered among her faculty the genial pioneer, Charles Edwin Bessey, who in the decade following 1870, gave a new and lasting impetus to the teaching of botany. Here undergraduate laboratory work in botany was first established, and the microscope as an integral part of laboratory work introduced. This, the first marked change in emphasis in General Botany, gave to biological science its chief impetus—"Leitfaden"—for the training of future biologists, and its distinctive claim to recognition in a cultural education. Upon this molding and directive character of the newly introduced laboratory work I wish to lay stress, since it will form one of the main themes for discussion.

Approximately a decade later there followed another innovation—Bessey's Textbook of Botany. In this he acknowledges his debt to the great master's "Lehrbuch," and makes available to the American student the several divisions of botanical science weighted with rare good judgment and skillfully coordinated through a single motive, namely function. A new epoch has been ushered in, a second change in emphasis —a shift from the taxonomic and external morphologic treatment of Asa Gray, to a study of the living plant through the recognition of the interdependence of structure and function.

Limitation of time will not permit me to enter fully into the historic development of the teaching of elementary botany, with its change in emphasis and its significance. However, one other, and perhaps the most important change from the viewpoint of the subject assigned me, must be considered, namely, the comparative morphologic. Dominated by morphologic tradition and fortified by a burning desire to prove evolutionary relationships, the practice of type studies and life histories assumes preeminence as another significant change. With conspicuous emphasis placed upon it, largely through the powerful personality that was its chief exponent, it has endured all too long for the best interest of botanical science.

In addtion to the above, which, for the purpose of discussion, may be considered as major phases in emphasis and significance, there are numerous minor phases that recent botanical discovery and changing interests and viewpoints have deemed essential to elementary instruction. That we

cannot go on and add by accretion is patent. The time for revision is now, and a most auspicious opportunity is offered by the program arranged by the Department of Botany of the Iowa State College of Agriculture and Mechanic Arts. Let us enter upon this task in a spirit of criticism and candor, realizing that as specialists in the several fields of botany, we must give and take, if our aim in organizing a new and better introductory course is to succeed. Should we as botanists, fail to meet and remedy the existing and growing objections to the heterogeneous courses now in vogue, largely the product of accretion—uncoordinated in the choice of subject matter and illogical in its relative emphasis—a revision from without is bound to come; all signs portend it.

A changing emphasis of vital importance is confronting us and is challenging us to meet it. From the addresses of several recently installed presidents of State Universities, we note a firmer insistence for better teaching, and an increased scrutiny into the nature of research. The change in point of veiw, on the part of our University executives, bridges the gulf between teaching and research, and this change in emphasis is prophetic of a brighter future for the real teacher. We must recognize that there shall always remain in our Universities those who make better teachers than investigators, and *vice versa*. These faculties are seldom equally strong in the individual; their proper balance in the University is imperative. A real appreciation of the important mission and influence of the teacher-investigator will not tolerate the disparity, so apparent in many of our institutions, between him and the investigator-teacher. Years ago at the luncheon table at one of our Association meetings, a gray haired pathologist, an international authority himself, was asked by one of a group of young doctors of philosophy, to name the man that had done most for the advancement of American pathology. Without a moment's hesitation he mentioned Doctor X. Thereupon the younger generation blared out in chorus, "Pray tell us, what has he done?" The answer was as quick and decisive, "Name me four pathologists over fifty years of age and of international reputation, that did not come under his tutelage."

The teacher must manifest an interest along some line of research and be actively engaged therein. Through it his mind is kept active and alert, and his teaching more dynamic. Through teaching the investigator reaps an experience in weighing values, and skill in coordinating the products of his research for the immature mind. By his profound knowledge, measured in terms of his ability to convey to the student in beginning botany, in simple and clear terms, the latest researches bearing on the subject under discussion, not infrequently fans the latent spark of interest into a boundless enthusiasm. To be able to free the great biological discoveries that affect human life and endeavor from the involved and erudite process that led to their discovery, and to translate them into simple terms, is the rare privilege of the real teacher. Perhaps the method used by L. Agassiz is worthy of emulation. I quote from Edwin E. Slosson, as follows:

"We are told that Agassiz required his students in every department to prepare 'first a monograph, second a scientific lecture, third a popular lecture, fourth a simple child's tale.' How many of our annual army of Ph.D.'s would pass the third and fourth of these intelligence tests? Agassiz had his reward in the dozens of devoted disciples who became the teachers of the next generation and in the thousands of young people who bear

his badge as they search forest and strand with curious eyes. But we need more men of the Agassiz type—and we seem to be getting fewer."

All of us have witnessed the fiasco of some University professor, not of the Agassiz type, who condescends to step from the high pedestal of research upon which recent University practice had placed him, to do a little teaching. Only recently I heard of a case where a professor of high standing was lecturing for a period of four weeks to a class in introductory botany, on the physical chemistry of the protoplasm—a changing emphasis whose significance, judged by proper standards, is failure.

The introductory course should be cultural rather than specific; that is, it should aim to present, concisely but accurately, those broader features of our science that will lead the student to a sympathetic understanding of his environment and the part that botany plays therein. Its content should be sufficient to furnish a broad but not detailed foundation to sustain the structure of intermediate courses (1) that the research student requires for the details of his extreme specialization; (2) that the teacher needs for the mastery of the more advanced work essential to give respect and authority; (3) that rewards the lay student who goes no further, with a wider and deeper conception of the world about him. To that end it is essential that the relation and dependence of botany to the other sciences be lucidly presented. Without it the plant as a functional unit in the complex interrelations between the lifeless physical world and the animal world, inclusive of man, cannot be interpreted. The beautiful functional adaptations of the plant and of its members—a product of the evolutionary process—that gave us the static plant as contrasted with the dynamic animal, are inspiring and fire the student with an enenthusiasm to go on. To prevent misunderstanding, let me state that by the beautiful functional adaptations, I do not mean the anthropomorphic teleology of the past, and still present in spots, but the modern teleology rooted in the profoundest discoveries of modern physics, chemistry, and biology, that compels us to recognize the thread of a plan running through the whole; that impels us to join with Millikan, Jeans, Evans, Haldane and others, in an attempt to outlive the undue emphasis that the rampant "mechanism" of the immediate past has imposed upon us.

To make the changes above suggested in the time allotted to our introductory course, it is necessary to relieve the already over-burdened existing course. That is a difficult task, and must be approached with frankness, and, perhaps I should add, with diplomacy. The choice of subjects to be deleted should be made through mutual accord between those holding opposing views, but always in a spirit that has, as its prime motive, a better botany.

Fortunately, both in England and America, botanists are fairly well agreed that—quoting from Bradley Moore Davis—"Morphology obviously can not ask for much more than the opportunity to serve the requirements of physiology since a knowledge of structure is basic to an understanding of function and life processes. The study of comparative morphology with the end in view of developing phylogenetic relationships is clearly impossible in so short and condensed a course." To this may be added that in the change in emphasis that leads us back to a study of the plant as a living unit whose chief purpose is food production, morphology will regain more than it has lost. Structure and function are inseparable and,

as coordinated fields of inquiry, lead to a common goal. That goal in elementary botany is to give the student, as a fundamental concept, the importance of the plant in that marvelous scheme of nature of which he forms a part. Intermediate in position between the mineral and animal kingdoms, it alone can bridge the gulf between the non-living and the living, and the definitely coordinated processes by which this is accomplished, in the broader aspects, furnish the central theme for emphasis. Around it all other botany—ecology, genetics, applied botany—must cluster, not as unrelated fragments, but as corollary to the main theme.

Looking toward the attainment of the foregoing ends, we examine next the laboratory instruction as the distinctive method of science. Here, too, a change in emphasis confronts us. Shall we, sixty years after its introduction, meeting in the same University city that gave it birth, lend our presence to its funeral obsequies? The answer emphatically is, No. It constitutes the keystone in the arch of scientific inquiry and method. It offers a form of training for which no substitute can be found in any other field of education, and, though not generally recognized, it is through the training it imparts, that scientist and layman, headed for widely different careers, may rise to greater heights. Nevertheless, a growing dissatisfaction is apparent; a dissatisfaction that has its origin not from within the scientific body to which we as botanists acclaim filial allegiance, but from educators and administrators.

Well might we pause to inquire into the motive that prompts such opposition. I believe we are not altogether guiltless of blame. The overemphasis placed upon research by University administrators, with the ready acquiescence of the investigator to join with the administrator in an unconscious disregard for good teaching, has made our laboratory work, in too many instances, a time-consuming hotchpotch. If science is to be recognized as rationalized and systemized knowledge it becomes our first duty to set the house in order.

The unrelated, and, in many instances, poorly chosen topics that form the basis for laboratory instruction, are left, with little guidance, to inexperienced assistants. The keystone of laboratory science is suffering from neglect and slow disintegration. It behooves us to change our course out of the backwaters, in which we now are drifting, into mid-stream that shall carry us back to a really scientific approach. If, heedless of the gathering opposition from administrator and educator, we fail to correct the evils for which we are in part responsible, there ultimately will remain for elementary instruction in science, little more than encyclopedic knowledge. And well may we inquire of an educational system that will allow such a catastrophe, as did Alexander von Humboldt, when, visiting the young and ambitious student Agassiz in the Latin quarter of Paris, and observing an encyclopedia displayed upon a shelf in his room, he asked—"Was thun Sie hier mit dieser Esel's Brücke."

BOTANY NOTEBOOKS

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In discussing the use of notebooks in botany, I am concerned chiefly with the general or introductory courses offered to and often required of the students in most of the large colleges and universities of this country. I take it for granted that these courses are intended to fulfill a general educational purpose rather than only to serve students who will specialize in botany or in allied sciences. In order to have some basis for making suggestions on methods of teaching such courses, even on what appears to be so minor an aspect as the taking of notes, I find it necessary first to point out in what respects much modern teaching of elementary science seems to me to fail to realize its general purposes. Much of this teaching is done in the secondary schools, and this also must be taken into consideration in attempting to formulate teaching aims and methods, because of the deep and often lasting impression made upon a young student by his first work in science; the habits of thought and the fragments of fact (or error) thus acquired are often decisive factors in his attitude toward his later work.

The effect of much of the teaching of science of today, especially that of the secondary schools, seems to be chiefly to inspire the beginning student with an awed respect for facts as such; for he comes to believe that education consists of memorizing them. Only by doing so can he secure the approval of his teachers and "pass the course." One has only to glance at some of the textbooks of general science or general biology used in the schools to realize that to their authors, and, necessarily, to the not very highly trained teachers who use them, science is an encyclopaedic collection of facts, and the object of the teaching of science to convey a smattering of information. The information, of course, may well be out of date before the victims of such teaching leave school; and only occasionally will any of it be the particular information which they will require in any given situation. They are no better equipped by such a smattering to obtain facts for themselves, to consider and verify facts, to draw conclusions from facts. Nor have they any means of knowing why this set of facts constitutes science, while another set of facts is labeled history or literature or something else.

The result is apparent in the attitude of many adults toward science and scientific fact. To our friend "the man in the street," who might perhaps be renamed "the man who reads the papers," science is of the nature of magic or religion, being based on authority or revelation. The prestige of the word science, and the childlike and often pathetic trust of the average man in anything labeled scientific must be interpreted in this light. The quack doctors and the advertisers of patent medicines, of tooth-paste, of cosmetics, and of thousands of other articles of commerce, are alert to take advantage of this attitude. Among the catchwords of the propaganda and popular misinformation with which we all are systematically pro-

vided, "scientific progress" commonly means an advance in technology, such as the invention and construction of a new machine; "scientific accuracy" seems to connote some sort of inhuman trickery, some faculty denied to the ordinary man. And the "science" invoked, with appropriate oblations, by modern business, becomes the newest and most potent of superstitions.

My reason for calling attention to the prevailing attitude toward science is to make clear my own point of view in formulating the purposes of science teaching. I believe that the value of education in science lies not in the facts accumulated (which may be obtained by any truly educated person from a technical manual or encyclopaedia), but in a mental attitude toward the unknown in general. Being educated is not merely learning how to be a receiving set for the brain-waves of others, but acquiring the ability to approach the surrounding world with some sort of intelligent inquiry and learning some of the methods by which it may be interrogated. If I may digress a moment, I am convinced that the distinction which is often made between the training of students who intend to enter college and that of those who will go no farther than the high school is overemphasized. Scientific habits of thought are valuable in almost all walks of life; the teaching of facts without any consideration of the methods by which they are acquired and judged is not only useless but misleading and often dangerous.

If it be granted that it is desirable for a student to acquire, in addition to information, certain habits of observation and thinking, it is obvious that individual laboratory work, while perhaps not absolutely essential, is of the utmost value. The demonstration of experiments or other material to a class by a teacher is not, in my opinion, a satisfactory substitute (though a valuable adjunct); individual work is necessary to enlarge the student's experience, to enable him to acquire personal habits and a relation toward the subject other than that which results from merely visual data. The mastery of any subject involves not merely the memorizing of certain statements of fact but an intimate emotional relation toward the acquisition of fact, a sense of achievement or of frustration, a recognition of difficulties and of the limits of the method. This is a principle which is recognized by every teacher of, for example, literature, when he prescribes individual readings. The value of laboratory work is precisely that the student finds in it an opportunity to do something himself besides look and listen.

It is, then, somewhat disturbing to find today a rather widespread hostility toward laboratory work, not only among the students themselves, but even among teachers. This arises, I think, partly from a lack of understanding of the educational aims which I have briefly indicated; and partly from resentment at some of the actual practices in laboratories. For it is a depressing fact that the laboratory routines of many courses in science fail to realize the benefits to be expected from laboratory work.

In many schools, for instance, the laboratory work may involve a few dissections, a few experiments, a few observations with microscopes; but most of the student's time is devoted to making entries in something called a "laboratory notebook." The chief value of this notebook is that it is later to be handed to the teacher to be "graded." It contains usually extracts from the textbook, often in the form of rigidly formulated "reports

of experiments," copies of figures and charts, and a few original illustrations of more or less artistic merit. Frequently many hours are spent at home making it as neat as possible, 'inking in" all of the drawings, and so forth. The proportions of different kinds of work vary, of course. I have seen notebooks from high schools which were entirely composed of painstaking copies of textual material, and which received an excellent grade. In colleges and universities equipment for original observation is more abundant; but the emphasis of many of the teachers is still on the notebooks, and any ostensible encouragement of originality of inquiry or thought is often a pious sham. It is considered sufficient, for instance, to compel the student to answer, in his notebook, certain printed questions on the material.

Such routines have originated in ideals both natural and praiseworthy. Scientific observation is of little value without some sort of record, and the features which constitute the value of the record are accuracy, thoroughness, clearness, permanency—all qualities the cultivation of which is valuable in any sort of an education. But the record, after all, is secondary. The photographic camera, no matter how exactly and permanently it can make records, cannot be substituted for the mind of the scientist. A poor sense of proportion in the teacher can ruin his laboratory teaching. Many a student who has taken general botany would have difficulty in explaining the difference between it and a course in art; so great was the stress placed by his teacher upon certain prescribed and required drawings, the making of which occupied almost all of the allotted time. I have at present several students in my classes who cannot be persuaded (so strong is the influence of previous schooling) to do anything else during their laboratory hours. They start drawing at the beginning of the period, long before they have understood the object which they are trying to represent, and occupy their entire time with making marks with a pencil on paper. They miss many of the chances for observation and discussion which other students are learning to use. Nothing I can say carries conviction, for they are deeply (if perhaps subconsciously) convinced that my only concern with them is to assign a grade to their productions. The cult of the notebook is really part of the worship of grades, which is probably the chief obstacle to good teaching in a modern university. It is perhaps mostly because of our preoccupation with the problem of grading large numbers of students that we have come to put the cart before the horse in our teaching. The record is something that may be judged and marked; we have too little time to inquire into the aptitudes and skill of the individual students to grade them on the extent to which they have learned to use their own faculties. If this is true, then it is time we recalled ourselves to our first duty as teachers. If something must be sacrificed, it must be the accuracy and ease of the grading rather than the value of the teaching.

It is something of a mystery to me that the product of any of the laboratory routines which I have briefly indicated should be called a *notebook*. Certainly the university student does not regard it as a repository for his notes. When he attends lectures, recitations, conferences and the like, he usually carries another notebook, which he really considers his own, and which is reserved for his eyes alone and for his future use. It is this notebook that he consults when preparing for examinations. If

any university teacher has not, by accident or otherwise, seen the contents of such notebooks, his knowledge of his own teaching methods is incomplete. The notes are usually a chaotic mass of garbled and disconnected statements, interspersed with attempts to copy diagrams and tables from the blackboard (I am speaking of the average earnest student). The diagrams are frequently unrecognizable, the notes often convey meanings (if any) exactly opposite to those intended by the teacher. Many of the errors in examinations may be traced directly to attempts to learn such notes, which so obviously represent the student's inability to abstract and his lack of time for a verbatim report.

Our courses in general science are still in part relics of small professional courses for students intending to enter scientific work. In the course of adaptation they have become curiously metamorphosed; they suffer from vestigial organs which are apt to become diseased. The laboratory and lecture notes, as often conceived, belong to days when the student's chief problem was to learn to represent what he saw; to days when perhaps textbooks were not as plentiful as they are now. The value of note-taking to the average modern student is twofold: in the laboratory he is likely to see more, to observe more carefully, if he is intent upon making a careful record; in all departments of his work he attains some mastery of the subject (as distinct from being merely a good listener) only when he is able to some extent to abstract and organize it; to single out the points worthy of emphasis from the mass of contributory detail; to make his statements clear, precise and free from error. The ability to take notes is, of course, neither inborn nor Heaven-sent; it must be acquired, and usually can be acquired only with the help of the instructor. The suggestions which follow embody some of my own attempts to help students to learn how to make records and to take notes which will be of some use to them in the ways I have mentioned. I do not see what other reason can exist for prescriptions or rules in making notebooks. The chief difficulty which I find in teaching elementary botany in a university is in the attitude of the students; that attitude which is compounded partly of the belief that science is a miscellaneous collection of facts to be memorized; partly of the assumption that laboratory work consists of making pictures and reports which will please the teacher.

It is not apparent why a student should have two notebooks, one for conferences and one for laboratory. This seems to me to stimulate in his mind a mistaken attitude toward the course as a whole. A student of mine was overheard to say the other day, when asked how he liked botany, "Oh, I like botany all right; but that laboratory—I don't care for that at all!" He thought he was taking two distinct courses. It is noticeable that many students regard the "lecture work" or the "quiz work" as the really important part of the course—the part upon which their grades mostly depend. This probably derives from the fact that a professor administers the lectures and may have written the textbook, while the laboratory is often in the hands of assistants, students only a few years ahead of the beginners themselves. But if the course is really one course, if the various parts of it are properly integrated, then any notes made, in any kind of work, may be expected to be of service in the whole understanding of the subject. In conferences and discussions, laboratory experiments and observations are described and their results and conclusions scrutinized;

in laboratory work the factual information provided by teacher or text-book is essential to an understanding of what is observed. It is essential to bear in mind that the beginning student is not a research worker and that nothing is gained by treating him as one; he is not engaged in the discovery of absolutely new facts which he can relate to a large body of very thoroughly understood knowledge. One notebook can house all the student's laboratory records, his lecture notes, his study notes, outlines and reviews. The notebook may then become an aid to the systematization of the work and the organization of the knowledge acquired.

The student enters college without any knowledge of the real value of note-taking or practise in doing so. This deficiency is natural to his age; indeed it is often found in graduate students, who may learn the value of careful records in research and in reading only by bitter experience. It is quite clear that the freshman needs guidance in this. He must be shown—as tactfully as possible—both when and how to take notes. I say tactfully because he must believe, from beginning to end of the process, that he is really *taking notes,* for his own benefit, and not completing a task to enable his teacher to label him with a grade.

In lectures and conferences the teacher must devote some time to telling the student what it is wise to put in his notebook, not only in general but in specific detail with reference to the matter in hand; and give him time to do it. It should be early and frequently pointed out that if he has previously studied the textbook he will be able to avoid wasting time and space duplicating statements of fact which may be found there. A fit subject for the taking of notes is, for example, organizing the contents of a cell, or the contents of a chapter. He may also put down questions to be looked up, common mistakes which should be avoided (especially statements which he has been previously taught to regard as correct, such as "that plants breathe;" or those which are true only when carefully qualified, such as "that living organisms adapt themselves to the environment"); and notes which will help him to single out for study the important points and more general aspects of any subject. Many of his notes must be, at least at first, dictated by the teacher. He should be taught some plan of heading pages, of indicating the subjects under discussion; this also must be clearly indicated by the teacher. I believe it was Dr. Johnson who first remarked that education had not yet taken advantage of the invention of printing. A lecture should not exhibit (as it often does even now) a lecturer repeating orally the information contained in his own textbook, while the students strive desperately to take down verbatim what they may find printed in that same book, under the illusion that they are thereby relieved of the necessity for studying it.

In the laboratory the student must be taught, both at the early meetings and from time to time thereafter, how it is possible to record some of the things he observes; by means of drawings, diagrams, tabulated records and the like. The protest against the making of drawings in laboratory has provoked various remedial suggestions, among which surely the most curious is to furnish the student with printed pictures of what he is to look at and to ask him to devote his time to labeling the pictures correctly. For the active inquiry which should be the object of every laboratory meeting is substituted a passive assent. It is not at once apparent why the teacher should not go a step further, and provide labels ready printed,

which may be affixed to the illustration by the student; the arguments against drawing apply also to labeling. The laboratory period could then be reduced, like the preparation of manuscript for a textbook, to exercise with scissors and gluepot. Another remedy, however, for the evils of drawing in laboratory consists not of doing the work for the student, but of teaching him what he can reasonably accomplish himself in the time available. Almost every experienced teacher knows that a very simple training will suffice to show an average student how to make drawings and diagrams which represent observed facts—or selected aspects of them —neatly and clearly, without undue expenditure of time, and without being "artistic."

The actual methods, and the standard of performance, best adapted to laboratory work vary with individual students. It is unfortunately necessary to impose some sort of rule, to give all students alike the same instructions on how to make drawings or how to tabulate the results of an experiment. Spontaneity is—or should be—the essence of laboratory work, and it is to be regretted that we are compelled, by the large numbers of students which we must teach, to set out certain definite "material" at stated hours on Mondays, Wednesdays and Fridays. It is perhaps worth while to explain this to the students, in the perpetual effort to lift them from their condition as slaves of a meaningless routine to one of active inquiry of the world about them. Fortunately it is still possible, even in crowded laboratories, to teach students individually, at least to some extent; a privilege not enjoyed to the same degree in most non-scientific courses. Every teacher can and should try to help each student to develop the methods best adapted to his abilities. What is valuable to one may be worthless to another. Instruction in making simple, clear diagrams is of value to all. But the student who can already draw rapidly, accurately and neatly should be encouraged to do so, while his neighbor perhaps may better express the same results in a much simpler diagram or in a written description. Elementary laboratory work has suffered from an overdose of printed manuals of procedure (so useful in more advanced work), which impose uniformity upon a very heterogeneous group, and stamp out initiative.

The principal idea which must be driven home in laboratory teaching, as this is related to the taking of notes and records, is that quality is more important than quantity. I know that in these days of volume production this statement amounts almost to treason. It is certainly a novel idea to most of my students, and one which they find great difficulty in taking seriously. But if our science teaching is to be of any value at all, the sort of thinking which it engenders must be accurate thinking, or it does more harm than good. It is more important to observe carefully and to record thoroughly than to reach the end of a given list of subjects. It is extremely difficult to make students believe this, or even believe that their teacher believes it. Perhaps the most effective means of conviction is the setting of examinations which are carefully and obviously planned to test the student's quality rather than his speed. In this matter of quantity as of method, great differences should be expected among individual students; even an inexperienced teacher can soon discern the different limits of their capabilities, and distinguish those who are naturally slow from those who are rapid but superficial and those who are more than ordinarily idle.

It follows from this that a considerable effort must be made to convince the student that he is not compelled to put anything in his notebook, nor is the amount limited in any way. The grading of notebooks, if it is done at all, must be done in such a way that the student has no doubt of this. It must be clear to him, from the teacher's comments, that all that is expected is that what is attempted should be done as well as possible. I believe, however, that it is a mistake to grade notebooks, for it always results in an unhealthy attitude of the student toward his notes. The notes, of course, may and should be examined in the laboratory as the work is completed. Criticism is of little value at any other time; for the purpose of laboratory instruction is surely to help the student to see and to record rather than to penalize him for not doing so. It is sometimes useful to have at first some sort of rule which will inspire the students to show their work to an instructor at frequent intervals during each period. Lecture and study notes may, of course, be brought to the teacher for criticism at other times.

In place of imposing upon the students a rule to be obeyed, a formula to be fulfilled, and constantly measuring the extent to which they conform, an effort should be made, as far as possible individually, to show the student that if he does not make any sort of careful record he loses the chance for valuable help and criticism, given during the class period or at least while the subject is still fresh. Informal tests—not necessarily written, not necessarily given to the entire class, but merely a part of the normal intercourse between teacher and student—help to convince the doubtful and the lazy that making notes is good policy. Many students, of course, will try experiments. Dazzled by the apparent freedom which is theirs, they seek at once to escape from all the conventions which they have hated through their school years. Sooner or later they work out for themselves some rational method and routine, usually not very different from what used to be prescribed in the course; but the experimentation itself which they undertake is of more value to them than any blind conformity to an established system. The function of the teacher is that of a guide, who can show the best method of attaining the desired end, and who can point out the virtues of system and simplicity. A plan of heading pages, a method of labeling drawings, the printing of labels rather than writing them in script—all such practices are valuable if, and only if, the students can be brought to see their value.

Laboratory work, of course, must be done and the records completed in the laboratory. It is one of the most distressing evidences of previous bad training that most students in my classes wish to make their first records on scraps of paper, and transfer them to a notebook later; their object being neatness and beauty in the finished product—and a good grade! The student must learn as early as possible how important, and how difficult, it is to separate one's actual observations from inference, speculation and explanatory matter. In fact, if he learns nothing else but this, his elementary science course is worth while. Any mechanical scheme which will set off, in the notebook, observed facts from other matter is to be encouraged. It is the custom in some laboratories to lock the notebooks up at the end of the class meeting, and to allow access to them only while the student is doing supervised work. This is presumably an attempt to enforce the above ideals; also to prevent cheating. If what

I have said so far is true, the effects of this practice, so far as the value of taking notes is concerned, are naturally disastrous. As for the prevention of cheating, if the students once learn that the notebook is not a grading device, there is very little tendency to "fake" it; they soon realize that they can cheat themselves only.

Some of these proposals will be condemned as "impractical." To this I can say only that nothing is impractical until it is tried and fails. Timidity is a regrettable trait in a teacher. When I contemplate the results of our science teaching, as evident in the general attitude toward science, I am tempted to believe that in trying to reform our methods of teaching we have nothing to lose and everything to gain. It is undoubtedly true that such methods as those which I am proposing miss many golden opportunities of grading the students. Personally, I am convinced that a multiplicity of grades, obtained "objectively" and computed mathematically, give no very valuable information about the students which a good teacher, working daily in contact with the students, does not find out for himself. But, whatever may be said about that, one thing at least is clear: the notebooks in a course in science must serve the students, not the students the notebooks.

AN APPLIED COURSE IN GENERAL BOTANY

L. W. DURRELL

From the Department of Botany, Colorado State College of Agriculture and Mechanic Arts

As you see on the program the particular part assigned to me is the teaching of applied general botany. I have been asked by the Committee to explain how we handle beginning courses and if you will bear with me I shall briefly sketch our procedure. Though I feel that before this group and at this institution it is like carrying coals to New Castle.

To begin with, I have close to 500 students in botany and the majority of these are beginners. It falls to my department to give the introduction to biologic science on our campus.

Times and conditions change, and the botanical goal of eight to ten years ago does not necessarily fit today. For years I lectured to all the freshmen, my main purpose being an effort to "sell" botany to them. I believed that the head of the department should be the one to make contact with the freshmen. Today, with the increased numbers, this can no longer be done even in a small school like Colorado Agricultural College.

At present we are using a modification of what is known as the Morrison Unit method with which you are doubtless familiar. It is something like this: The subject is divided into logical units such as leaf structure and function, root structure—water supply and water intake, seed structure and seed germination, variation and heredity. Each instructor has complete charge of a group of students.

When a unit of study is under consideration the subject is presented first by either a formal lecture, discussion, or text or reference assignment. This may be preceded by a pretest to see how nearly ready the student is for the next lesson.

Recently Nicholas Murray Butler said that the lecture should have gone out of existence at the time of Gutenberg. You may not agree with me, but I still think that there is a place for the lecture. Many people are more ear- than eye-minded and in some cases the verbal presentation speeds the information process—at least, the lecture can analyze and interpret.

After this so-called presentation should come, according to the formula, "Assimilation." This may consist of laboratory examination of specimens in hand, notebook drawings, and reports consisting of special assignments, use of demonstration microscopes and the making of life cycles of organisms studied.

The "Assimilation" period should be followed by "Organization," this being a discussion of the unit through leading questions; and finally by a written summary of the important facts of the unit of study which is being considered. Lastly, let us have a written or oral test, over the subject. I would like, if I dared, to add to this formula a comprehensive, coordinating examinations at either the end of the year or the end of the four years.

Now a few "stunts" may be added to show the students phases of practical botany such as trips to the research laboratories or to the state seed laboratory; lectures on poisonous plants, on weed control or on important plant diseases by specialists on the staff. This all works for interest and emphasizes the worthwhileness of botany.

I have noticed that students appreciate worthwhile, hard courses—now don't misunderstand me, I do not mean by a hard course an obscure course.

Now all I can say for the above scheme is that it seems to fit into our organization, covering three two-hour periods per week, 30 students per section. My associates prefer it to any arrangement we have yet tried. It combines a minimum of lecture with the other work. Though all instructors are required to cover the same general ground, and each has a syllabus and tentative time schedule of the course, they have perfect freedom as to how they present the subject and are encouraged to inject into it anything of interest, botanical or otherwise, that will put the work across.

Furthermore, the work can be coordinated—there is none of the old discrepancy between lecture and laboratory. In fact, the subject should be considered as an entity—the laboratory as preparation for discussion or quiz.

Now, after patiently hearing me so far, you will probably say, "Why, that is not applied botany." Of course not! As I understand it, applied botany is Agronomy, Horticulture, Forestry, Plant Pathology, which are studies for consideration of older students. I wish to take issue with this view of applied courses in botany or any basic science—training only to the end of the applied or practical. The curse of agricultural training is such courses as Agricultural Botany, Agricultural Chemistry, Agricultural Physics—the wind tempered to the shorn lamb. A prominent agronomist told me last year, "Let my students have three years of science; I will teach them their agronomy in the last year." This statement may sound strange from the lips of an alumnus of an agricultural school, from an employee of an agricultural experiment station.

But let us pause and take an inventory of the graduates of land-grant colleges. While such schools were primarily established as trade schools and their graduates were intended to go back to farms or into industry, we find that of late years only a small percentage do so. Many of them are in positions where they should be leaders in agricultural thought. But do they find themselves trained for this? Unlike some of our older scientists they never have developed inquiring minds—in many cases getting a degree after focusing attention on some minute sphere of interest and know little of the broader channels. This situation arises because we teach applied botany or chemistry too early in the course. No consideration of the humanities, no contact with the philosophies and thoughts of the great minds of the past, no broad preparation on which to stand when in positions of responsibility.

Nationally we have made the mistake in education of making a fetish of schooling; thinking it would solve all human problems, that it could take the place of wisdom.

Other points of consideration which have forced themselves upon my notice: We have produced too many technicians and specialists—there is scarce place for them; we have been guilty of encouraging and bribing

students with fellowships and scholarships until they have had more eye for the stipend than interest in the work. A change has come under stress of depression; we are analyzing, searching for the verities; we are finding that science and art may be enjoyed for their own sake; we are getting away from professionalism. People are showing interest again in the botany of the fields, in popular science, in art, in doing things and making things. Not all want practical or professional botany. Are we to teach applied botany to such? If we wish to attract, to enlist, we should design a plan for botanical teaching stressing interest over all else. Employ enthusiastic teachers; and here may we say a word on the duty of administrators to see that such men do not stultify themselves with the deadening system of quarter after quarter of continuous teaching, but have opportunities for travel and study, that they may bring back to their classes new and interesting thought. We need artists at teaching—not artisans.

Put your research laboratories in the front of the building where they attract attention and arouse curiosity. Further, we cannot regiment either the students or the instructors—they will not click alike any more than the clocks of Charles V. Let the instructor be responsible and use his own head.

Most important, young people like to do and to imitate, hero worship, if you please. Here we find the key to success in botanical teaching, not a system—but contagious achievement. Wherever you find an interesting scientist you find back of him a C. E. Bessey, an M. B. Thomas, or an L. R. Jones, the personality of men of enviable achievement—men of broad culture, students of the humanities as well as of their chosen fields, men who have made life a thing of beauty, who have learned the Art of Living.

May I close with a paraphrase of Eucken of a passage from Aristotle:

> "But excellence arises into distinction by the working out of the difference between beauty and utility or that which pleases in itself and that which is valued as a means to something else. Who ever makes utility the chief consideration is guilty of an inner perversion of life, for the service of utility continually directs activity to outward alien things while with all the supposed advantages the soul is left inwardly empty. It is the business of a free and large minded man, everywhere to seek beauty rather than utility."

A USE OF THE PLACEMENT TEST IN THE TEACHING OF GENERAL BOTANY

WILLIAM A. KREUTZER[1]

From the Department of Botany, Iowa State College

An investigation of the use of the placement test as a method for sectioning general botany students into groups of comparable ability was initiated in the fall of 1934[2]. The rôle of the placement test in segregating students into "fast" and "slow" sections is well known in secondary schools, but such is not the case at the college level. It is recognized that different departments in dealing with large numbers of students have attempted segregation on one basis or another, but the results have seldom been permanently recorded. This is especially true in the field of this particular study. Although some work has been published on the use of the placement test at the college level, limited space precludes any review of the literature.

Whether general botany is most successfully taught to mixed groups or to groups in which students are of relatively equal ability is a question which may have a definite bearing on the most advantageous method of teaching this subject. Further, it may well be that by the inauguration of such groups individual student progress would be accelerated.

The initial step in the study of this problem concerns itself with a determination of the diagnostic value of a placement test using two examinations and the instructors' observations as criteria.

METHOD

A placement test was given to all students classified in botany 101 at their first regular class meeting. It consisted of a permanent record sheet and eight questions on separate sheets. The test was designed to measure the ability of the student to read intelligently, to set up an original experiment, to demonstrate the truth or falsity of any given hypothesis, to judge intelligently, to analyze data, to draw inferences, and to arrive at logical conclusions based on experimental results. In the subject matter of the questionnaire it will be observed that no previous knowledge of the field is necessary, all the essential facts being incorporated. (See addenda.) Questions were distributed separately to the students, care being taken to observe the time limit allowed as indicated on the top of each sheet. At the end of the specified time, all sheets pertaining to any one question were collected before another was distributed.

In order to obtain as much uniformity in grading as possible, each instructor was assigned the task of correcting all the answers to one question. Each question was scored on the basis of 100 points, the average of the eight questions constituting the student's grade. The instructor was then asked to turn in a written report which stated the manner in which he scored the particular question assigned to him. On the basis of the grades obtained in this test, the students were next sectioned into high, intermediate, and low groups, the presence of an intermediate group, depending upon the number of students meeting at any one hour of the day. For example, if there were over 60 students meeting at any one time, three groups were set up, whereas, if there were less than 60, an intermediate section was omitted. Since our class rooms are equipped for only 30 students, the numerical basis for segregation varied with the group in question. It is recognized that the individuality of the instructor is one of the factors influencing student progress. For this reason any instructor teaching three sections was given a high, an intermediate, and a low group. If the instructor taught only two sections he was given a high and a low, or a high and an intermediate section. The apportionment of sections is shown in table 1.

The next step was to determine how well the placement test had accomplished its purpose. In order to decide this a general examination was given at the completion of the first unit problem of the course (midterm) and at the end of the school term. These examinations were made up by the entire teaching staff. Both examinations were designed to measure the information acquired by the student, but they stressed primarily the ability of the student to actually use the information acquired. An examination other than the general midterm was given to one high and two low sections because these three groups met two days after the midterm examination was first used. For this reason the midterm examination grades of the students in these sections were not used in the study. Answers to any one question in both examinations were scored by one instructor to insure the same uniformity in grading comparable to that obtained in the placement test. All sections took the final examination simultaneously. The midterm and final examinations are listed in the addenda, complete as given.

EXPERIMENTAL RESULTS

A total of 287 students took the placement test. Of this number, 13 dropped the course before the midterm examination, five dropped out after the midterm examination, two failed to take the final examination, and six were not included in the final calculations. This left a total of 261 students whose grades for the midterm and final examinations were used. Of this group 77 students were given a midterm examination other than the general examination, consequently only 184 midterm grades are considered. Only the grades of those students taking the placement test and final examination are used in analyzing the diagnostic efficiency of the placement test.

The average grade for each examination per section is shown in table 1. The designation of the group and the number of students per section are also given. The instructor in charge of the section is indicated by letter only.

TABLE 1. *Section averages for the placement test, midterm, and final examinations*

| | | | | Average grade | | |
Group	Section	Instructor	Number students counted	Placement test	Midterm exam.	Final examination
1. Mon. Wed. Fri. 8-10 a. m. sections	High Inter. Low	A B C	19 24 24	81.4 67.8 54.5	71.4 60.4 56.3	60.1 57.1 50.7
2. Mon. Wed. Fri. 10-12 a. m. sections	High Inter. Low	D C E	32 17 18	74.5 63.6 48.8	74.5 72.6 61.8	57.8 62.6 50.8
3. Mon. Wed. Fri. 1-3 p. m. sections	High Inter. Low	B F A	22 29 21	81.3 68.2 49.7	...... 66.5 65.8	64.1 63.6 57.0
4. Tu. Th. 1-3 p. m.; Sat. 10-12 a. m. sections	High Low	C D	27 28	78.6 56.0	....	65.9 58.2

In all cases the average midterm examination grade for each section is comparable to the designation of that group. For example, it is evident that the average midterm examination grade for any one high section is higher than the average midterm grade of the intermediate section of the same group. In the same manner, the intermediate midterm examination grade of any one group shows superiority over the average midterm examination grade of the low section in the same group. The high section in group 2 shows an unexpected drop in the average final examination grade. This drop is known to be due chiefly to factors which were beyond control. There is evidence here, as shown by the final examination averages, that the placement test may not be particularly diagnostic for the segregation of intermediate groups.

In summarizing these results, the test averages per unit group as shown in table 2 indicate that the placement test was diagnostic for high and low sections, and to some degree for the intermediate sections.

TABLE 2. *A comparison of the examination averages for high, intermediate, and low sections*

| | Placement test | | Midterm exam. | | Final examination | |
Section	Number students used	Grade average	Number students used	Grade average	Number students used	Grade average
High	100	78.4	51	73.3	100	61.9
Intermediate	70	66.9	70	65.8	70	61.1
Low	91	52.7	63	61.0	91	54.4

The midterm examination averages indicate that the placement test was diagnostic for all three groups, however, the final examination aver-

ages for the high and intermediate groups show only a slight difference. This apparent overlapping of the two groups may be accounted for, at least in part, by the previously mentioned, unexpected low final examination average obtained by the largest high section as shown in table 1.

INFLUENCE OF THE EXAMINATION GRADES OF UPPER AND LOWER CLASSMEN ON THE SECTION GRADE AVERAGES

In the high sections 74.9 per cent of the total number of students were freshmen, 16.1 per cent sophomores, 6.0 per cent juniors, and 3.0 per cent were seniors. In the intermediate sections 71.4 per cent of the students were freshmen, 24.3 per cent sophomores, 4.3 per cent juniors, and there were no seniors. In the low groups 82.6 per cent were freshmen, 14.1 per cent sophomores, 2.2 per cent juniors, and 1.1 per cent were seniors. The majority of the freshmen were in the low and high sections, the bulk of the sophomores were in the high and intermediate sections, while the greater number of juniors and seniors were in the high groups.

Although the majority of the students in all sections were freshmen, the scores primarily made by sophomores undoubtedly influenced the section averages for the midterm and final examinations. Grade averages for upper and lower classmen are shown in table 3.

TABLE 3. *Average grades per class for the three examinations given*

Class	Placement test		Midterm exam.		Final examintion	
	Number students used	Average grade	Number students used	Average grade	Number students used	Average grade
Freshmen	200	65.4	150	65.4	200	58.7
Sophomores	46	67.5	27	73.6	46	61.2
Juniors	11	73.5	5	68.8	11	62.4
Seniors	4	71.9	2	75.7	4	64.6

A general tendency for upper classmen to make better scores than lower classmen is indicated. This result was expected in as much as it tends to verify the work of Miss Etta Gene Victor[3] who showed in her thesis that, as a rule, upper classmen tend to show more ability than do lower classmen.

Because of the small number of juniors and seniors as compared with freshmen, it is quite possible that the average grades as shown by the two former classes are no true criteria of these groups. The average grades for the final examination, however, show the expected gradation.

DISTRIBUTION FREQUENCY OF EXAMINATION GRADES FOR HIGH, INTERMEDIATE AND LOW SECTIONS

The data which are represented graphically in figures 1, 2 and 3 show the distribution frequency of examination grades for each group of sections. In the histograms of figure 1 the scores obtained by the students

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[3] Context and presentation of general botany at Iowa State College, Etta Gene Victor, Unpublished Thesis, Library, Iowa State College, 1933.
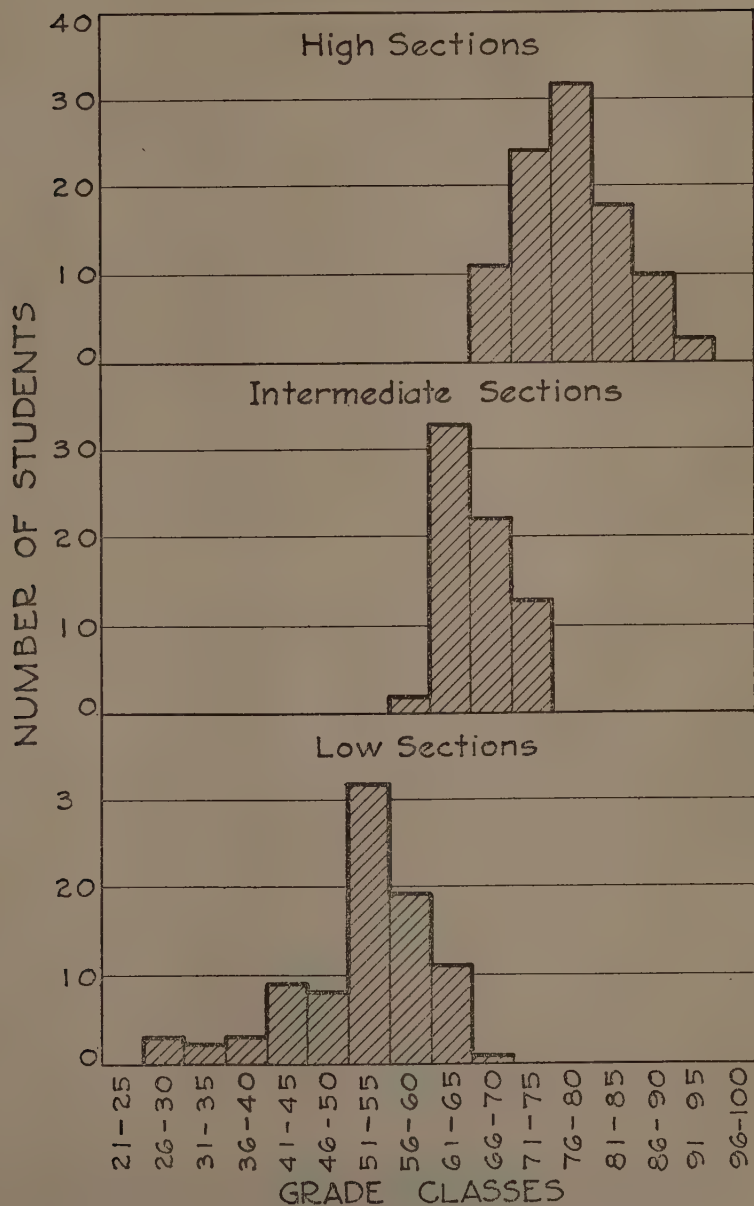
Fig. 1. Histograms showing the distribution of grades in the placement test.

in the placement test which served to segregate them into sections are shown according to these sections. A certain amount of overlap of grade classes in the three groups is apparent. The reason for this, as has been previously explained, is that the basis for sectioning varied with the group in question, depending entirely on the number of students meeting at any one time.

As shown in figure 1, the low sections include a greater number of grade classes than did either the high or intermediate groups. Figures 2 and 3 show an increase in the number of grade classes per group, especially in the cases of the low and intermediate sections. In addition, figure 2, in bringing out the distribution of grades for the midterm examination, shows a tendency for the total number of student grades to be more uniformly distributed in the grade classes represented. This effect is more noticeable in the intermediate and especially the low groups. The most apparent point shown in figure 3 is brought out in the final examination grade distribution for the high sections. A greater grade class spread is evinced for this group. In addition, there appears to be no significant difference in grade distribution between high and intermediate sections. The histograms in figure 2 show more correlation with those in figure 1 than they do with those in figure 3. This suggests a greater correlation between the placement test and the midterm examination than between the placement test and the final. The examination averages shown in tables 1 and 2 seem to corroborate the same tendency.

RELATION OF THE FINAL EXAMINATION GRADES TO SECTIONAL DISTRIBUTION

Of the total number of 261 students whose grades were used in this study, 38.3 per cent were in high sections, 26.8 per cent in intermediate sections, and 34.9 per cent in low sections. These percentage groups are considered as upper, intermediate, and lower brackets. In order to determine what percentage of the students obtaining high scores in the final examination were actually in high sections, final examination grades representing the upper 38.3 per cent were studied in relation to the sectional distribution of the students receiving them. This same type of analysis was made for students obtaining final examination scores representing the intermediate 26.8 per cent and the lowest 34.9 per cent.

The results of this study are shown in table 4.

TABLE 4. *Relation of the final examination grades to the sectional distribution of the students obtaining them*

Final examination grades	Distribution of students according to section		
	High	Intermediate	Low
		Percentage	
Highest scores (38.3 per cent of the total)	47.0	34.0	19.0
Intermediate scores (26.8 per cent of the total)	34.3	28.6	37.1
Lowest scores (34.9 per cent of the total)	31.8	18.7	49.5

From table 4 it is evident that all but 19.0 per cent of the students obtaining the highest scores in the final examination were in the high and

intermediate groups. The sectional distribution of those who ranked intermediate in the final examination indicates an expected uniformity. Although almost 50 per cent of the students receiving the lowest scores in the final examination were in the low group, there is a higher percentage in the high groups than expected. This latter fact probably can be accounted for by the relatively low final examination averages as shown by some of the high sections (table 1).

GENERAL OBSERVATIONS OF THE TEACHING STAFF

According to the concensus of opinion of the entire teaching staff, based on direct observation, the groups segregated by the placement test showed marked differences in ability. In general, the high sections showed considerably more initiative and independence as a unit than did the other two groups. In addition, the high sections were more versatile in the application of botanical principles and seemed to be capable of doing a greater amount of work. The intermediate groups although not showing the initiative and independence of the high sections, seemed fully as thorough in their undertakings. Low sections, although willing, appeared to be slower in general comprehension and lacked initiative as a group to a marked degree. In teaching a low section, the instructors report the requirement for considerable simplification, and a need for the expenditure of more time in the study of any one topic. Much more repetition was necessary with the low groups than with either the intermediate or high sections.

Although recognizing the need for further study of this nature, the teaching staff is of the opinion that this attempted segregation of students into groups of relatively equal ability brought about a noticeable improvement in the teaching of general botany.

SUMMARY

An investigation of the use of the placement test as a method for sectioning general botany students into groups of comparable ability was begun in the fall of 1934. A placement test was given to all students classified in botany 101 at their first regular class meeting. From the scores made in this test, they were segregated into high, low and intermediate sections.

A general examination was given at midterm and at the end of the quarter to determine the diagnostic value of the placement test. The midterm examination averages indicate that the placement test was diagnostic for the segregated groups. The final examination averages although showing that the placement test was diagnostic for high and low groups, tend to point out that intermediate sections cannot be segregated with accuracy. Examination grades on a distribution frequency basis tend to point out that there is a greater correlation between the placement test and the midterm examination than between the placement test and the final examination. As determined by the placement test a greater number obtaining the highest scores in the final examination were in the high sections and the largest group of students obtaining lowest scores in the final examination were in the low sections. For practical purposes the placement test was diagnostic.

An analysis of student classes represented in the sectioned groups indicates that the majority of freshmen were in low and high sections, the
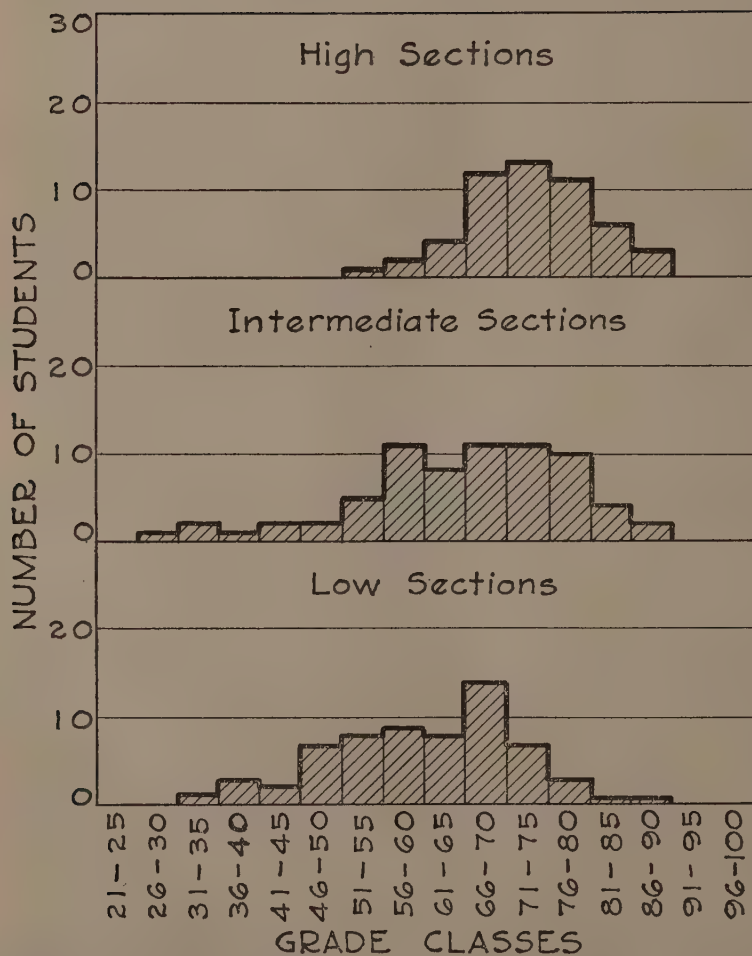
Fig. 2. Histograms showing the distribution of grades in the midterm examination.

bulk of the sophomores were in the high and intermediate groups while the greater number of juniors and seniors were in the high sections. Lower classmen, as a group, did not obtain as high scores in any of the examinations given as did the upperclassmen.

ADDENDA
Placement Test
Record Sheet

Classification (check one)

Freshman............Sophomore............Junior............Senior............

Proposed major...

Experience:

I have taken a course in (check)

| High school botany | .... | College botany | .... |

High school botany   ....   College botany   ....

" " biology ....   " biology ....

" " zoology ....   " zoology ....

" " chemistry ....   " inorganic chemistry......

" " physics ....   " organic chemistry........

" physics ....

List any other science course or courses that you have taken in the past.

Problems.

1-5 min.

A mature corn plant, including stem, leaves, grain, cob, and roots weighed 2707 grams. After the plant was dried, it weighed 835 grams. What percentage of the green plant by weight was water? (453 grams= 1 pound).

In solving the above problem please show all of your calculations and reasoning in the space provided below.

2—8 min.

One of the most fundamental differences between a green plant and an animal is that a green plant can manufacture its own food while an animal cannot.

Read over the following statements selecting any which you believe to be correct, giving your reasons in the space provided. If you do not believe that a statement is true, leave the space blank.

a. Plants can live in a world where there are no animals.

Reasons: ....

....

b. Animals can live in a world where there are no plants.

Reasons: ....

....

   c. Plants cannot live in a world where there are no animals.

      Reasons: ....

      ....

   d. Animals cannot live in a world where there are no plants..

      Reasons: ....

      ....

3—7 min.

All living things absorb oxygen and give off carbon dioxide. This process is known as respiration. Even seeds, since they are alive, respire.

The data represented in the following table show the effect of moisture on the respiration of two kinds of wheat seeds. Respiration is measured here in milligrams of carbon dioxide given off. (A gram is a very small unit of weight, while a milligram is equal to one one-thousandth of a gram.)

Percentage of moisture in the kernel	Milligrams of carbon dioxide given off every 24 hours for every 100 grams of seeds	
	Wheat No. 1	Wheat No. 2
11	0.53	1.00
12	0.70	1.10
13	1.00	1.58
14	1.56	3.00
15	3.27	5.80
16	7.58	11.80

In the space provided below, briefly explain the meaning of the above table.

4—10 min.

Let us assume that you are to spend the rest of your life on an imaginary island on which there are neither animals nor plants. For the sake of our illustration let us assume that any plant will readily grow there. You are allowed to take the seeds of six different kinds of plants with you, and these plants must supply your needs for the rest of your life.

Please list the six plants you will take in the spaces provided below, giving in each case a brief reason for your choice.

   1. Plant..

      Reason for choice: ....

   2. Plant..

      Reason for choice: ....

   3. Plant..

      Reason for choice: ....
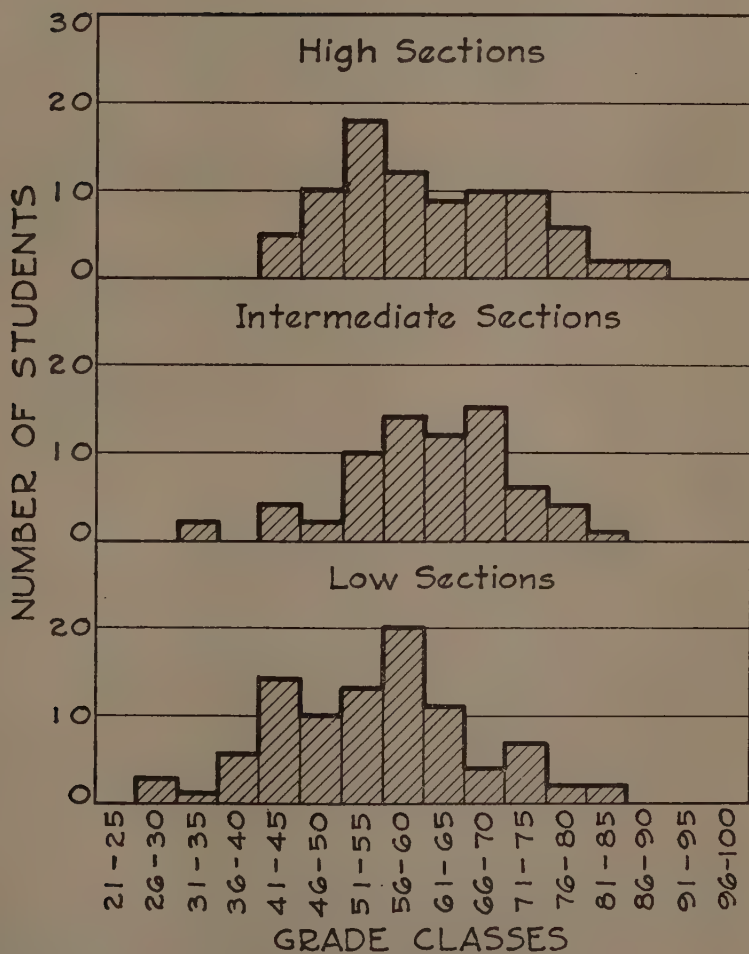
Fig. 3. Histograms showing the distribution of grades in the final examination.

4. Plant...

    Reason for choice: ....

5. Plant...

    Reason for choice: ....

6. Plant...

    Reason for choice: ....

5—7 min.

    Plants constantly give off water in the form of vapor just as any moist material does when it is exposed to the drying effect of the air.

    Two men who were interested in the amount of water lost from plants, made an intensive study of this subject. Here is a table taken from some of their work.

    The total water loss in a 24 hour period is considered as 100 per cent.

Kind of plant	Number of plants used	Average percentage of total water loss for a 24 hour period	
		During daylight	During darkness
Wheat	50	96	4
Oats	52	94	6
Rye	57	95	5
Sorghum	47	95	5
Alfalfa	52	97	3
Pigweed	45	97	3

    In the space provided below, state briefly what the contents of the above table indicate.

6—7 min.

Read these facts carefully:

    Starch when treated with iodine gives a blue color. When saliva is mixed with starch and left for a time, the mixture no longer turns the iodine solution blue.

    Using only these facts which you have been given, *check* the statement which appears most logical in the light of the above facts.

a. Saliva turns starch to sugar.....................

b. Iodine turns starch to sugar.........................

c. Saliva will not mix with iodine.................

d. Saliva produces changes in the starch......................

e. Saliva turns iodine blue.....................

f. The color of saliva destroys the blue color.....................

    Adapted from: Ralph W. Tyler, *Measuring the Ability to Infer,* Educational Research Bulletin IX. No. 17: 475-80. Ohio State University. 1930.

7—7 min.

Many years ago, an Englishman named Priestley placed several mice under a bell jar (a large glass jar) which was then sealed in such a manner as to exclude all the outside air. He observed that the mice died in a very short time.

Later, he placed more mice under the same bell jar, and, in addition, a potted plant. He then resealed the jar. He noticed this time that the mice lived with apparently no discomfort during the first day, but died during the first night. Priestley repeated this experiment several times, and always obtained the same result.

Assuming that you know no more about botany than Priestley did, put yourself in his place and enumerate briefly in the space provided below the conclusions that you would draw from the results of this experiment.

8—8 min.

Read these facts over carefully:
   a. The leaves are the food making organs of a plant.
   b. The food manufactured by the leaf is sugar.
   c. A great deal of this manufactured sugar changes to starch in the leaf.
   d. Iodine when added to starch turns it a dark blue.

With the above facts in mind describe briefly how you would set up an experiment to show whether or not light is necessary for a plant to make food.

Midterm Examination

1 a. Why are experiments dealing with respiration commonly performed with germinating seeds rather than leaves or whole mature green plants?

1 b. Read the following statements and place a check mark opposite the one which seems the most logical explanation: Practically no carbon dioxide is set free from the palisade and spongy tissue of the leaf on a bright day because:

   a. The sun is shining.....................

   b. The plant is transpiring.....................

   c. Carbon dioxide is being used in the process of photosynthesis......

   d. The plant is respiring.....................

   e. The carbon dioxide goes into solution in the water in the leaf........

2. Using the code below, write the appropriate number or numbers in each blank space.
   If true of *transpiration,* write *1.*
   "   "   " *photosynthesis,* write *2.*
   "   "   " *respiration,* write *3.*
   "   "   " *none of the above,* write *0.*

........requires the presence of free oxygen.

........energy is stored.

........water vapor is given off.

........occurs in green plants.

........oxygen is given off.

........starch is synthesized.

........takes place only in presence of light.

........receives its energy from the sun.

........is the same as breathing.

........is the same as evaporation.

........energy is released.

........the reaction $6CO_2 + 6H_2O = C_6H_{12}O_6 + 6O_2$ occurs.

........gives off material through the stomata.

........the reaction $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ occurs.

........occurs in plants lacking chlorophyl.

........takes place in all plant cells.

........gives off CO_2 at night.

........takes place in plants only.

........chlorophyl is necessary.

........indicates the presence of plant life before animal life on the earth.

3. What is your understanding of the following terms?

1.	chlorophyl	11.	epidermis
2.	chloroplasts	12.	vacuole
3.	palisade tissue	13.	petiole
4.	stoma	14.	midrib
5.	photosynthesis	15.	stipule
6.	cell	16.	pinnately compound
7.	starch grain	17.	cytoplasm
8.	leaf	18.	bulliform cell
9.	guard-cell	19.	resin canal
10.	leucoplast	20.	protoplast

4a. A leaf of hen-and-chickens dried up in 12 hours when the epidermis was pulled off. Another leaf left with the epidermis intact, with the stomata functioning and open most of the time, did not dry up in a week. Why?

4b. Trace a molecule of CO_2 from the air to the final product utilized by the plant. Assuming it to be used in respiration, trace it back into the air.

Final Examination

1. Why can perennial weeds like Canada thistle be eradicated by frequent and continued cultivation?

2. Read each of the following statements. If you agree, place a "+" in the place before the number; if you disagree, place a "—" before each number. If you do not know, leave the space blank. *Do not guess.*

.... 1. Sugar is manufactured in the xylem.

.... 2. Sieve tubes have no end walls.

.... 3. Transpiration is the release of CO_2 by the plant.

.... 4. The tendency of substances in solution to concentrate at one point is called diffusion.

.... 5. No carbohydrate can be made by plants in darkness.

.... 6. Parenchyma is frequently used for food storage.

.... 7. Pith is composed of sclerenchyma and cortex.

.... 8. The act of placing pollen on the stigma is called fertilization.

.... 9. The endosperm nuclei have the 3X number of chromosomes.

........10. Diffusion through a membrane is called respiration.

........11. Water is a medium for transportation of foods in plants.

........12. Photosynthesis occurs in all living cells of a plant.

........13. The vacuole is a vacuum in the cytoplasm.

........14. A meristem is the main stem of a plant.

........15. Provascular or procambium strands give rise to xylem and phloem.

........16. Division of cambium cells increases the length of the stem.

........17. Collenchyma cells have uniformly thickened cell walls.

........18. A tangential section is a longitudinal section.

........19. A rhizome is a modified stem.

........20. Secondary roots arise from the epidermis.

........21. Cell division is a continuous process.

........22. Food storage is the principal function of the xylem.

........23. Increase of diameter in woody stems is caused by division of xylem cells.

........24. Energy is released during respiration.

........25. All monocots are grasses.

........26. Chromosomes maintain their individuality through the division process.

........27. Starch is composed of water, carbon dioxide, and chlorophyl.

........28. The leaf is the principal organ of food manufacture.

........29. The potato is a swollen root.

........30. 2x chromosomes are found in gametes or germ cells.

........31. Phloem conducts water and dissolved minerals.

........32. Reproduction by cuttings is asexual or vegetative.

........33. Oxygen is a by-product of photosynthesis.

........34. The pollen tube is a gamete or germ cell.

........35. The essential parts of the flower are the stamens and pistil.

........36. Most monocots are herbaceous plants.

........37. The by-products of respiration are CO_2 and O_2.

........38. Cell division occurs most rapidly in the root cap.

........39.   Roots normally have no pith.

........40. Four pollen grains are produced following one reduction division in the pollen mother cell.

........41. Chromosomes carry determiners (genes) for hereditary characteristics.

........42. Chlorophyl turns into starch when exposed to light.

........43. An ovule following fertilization becomes a seed.

........44. The pollen tube nucleus fertilizes the polar nuclei.

........45. Seeds that have been preserved 5000 years will sprout.

........46. Wheat matures more rapidly in Alaska because the days are longer.

........47. The rind of a corn stem is made up of xylem.

........48. All living cells respire.

........49. Meristematic tissue is tissue in which the cells may divide.

........50. Leaves originate as outgrowths of the provascular strand.

3. Edit: Make corrections in matters of fact, logic, and English.

Growth, in its broadest sense, means enlargement. In plants it is accomplished in three steps, cell-division, cell-enlargement, and cell-differentiation. Since all plants are made up of cells the size of the nucleus becomes significant. The cell is dependent upon its surface for its very existence; that is to say, all foods or raw materials must enter the cell through its surface and waste products must be eliminated by the same path. However, geometry tells us that the surface of a sphere increases as the square of its diameter, while the volume increases as the cube of the diameter. Thus we see that the larger the cell the more

surface it has proportionately if it is spherical and the same facts hold true, to a less degree perhaps, for cells of other shapes. This proportion of surface to volume then is one of the chief factors in determining the shape of the cells.

Let us now look into the stages of plant growth. Cell division is the first step and takes place only in plant meristems; that is, in tissues which are composed of small thin-walled cells with proportionately large nuclei and abundant cytoplasm. Cell division apparently is initiated in the nucleus. The nucleus divides by meiosis in such a manner that each daughter-cell receives representative halves of the chromatin materials. These meristems are to be found in three principle regions, the stem-tip, the root-tip and the cambium. After division has ceased the cells enlarge and this enlargement is confined to regions adjacent to the regions of cell division. In the stem-tip and in the root-tip the region of enlargement is known as the region of elongation and lies behind the region of cell-division in each case. After enlargement the cells take on the modifications which fit them for their special functions, that is, they differentiate. In the cambium the region of enlargement is on both sides of the region of cell division and are confined to a narrow band of tissue inside the cambium in the phloem region and outside the cambium in the xylem. These facts being true, the elongation of the stem occurs only by extension of the stem-tip, and of the root by the extension of the root-tip. The cambium in a like manner is responsible only for growth in diameter.

4. Give experimental evidence that helps you to explain the following observations:
   (a) leaf-eating insects bring about a decrease in the yield of fruit on trees.
   (b) waste liquors (brines) from a salt factory destroy vegetation near the factory.

5. Some water, soil, a fish and some green algae (Spirogyra) were placed in a sealed glass vessel. The latter was placed in the class room when it was light during the day and dark at night. Although the vessel was kept sealed, the algae and fish were thriving at the end of a six-month period. On the basis of what you know about fundamental processes in plants and similar processes in animals, explain the situation in the sealed vessel by filling in each of the following blanks.

a. Carbon dioxide is used by the algae in the process of.............................

b. The process of .... is taking place in all living cells of both the fish and the algae.

c. When carbon dioxide and water unite in the algae, ... is formed and .... is liberated.

d. Oxygen is used by the fish in the process, ...

e. Oxygen is used by the algae in the process, ...

f. Carbon dioxide is a by-product in the algae in the process of ....

g. The soil in the vessel supplies .... used in making food.

h. The direct source of energy utilized by the fish is ....

i. The algae take in the raw materials by the process, ...

j. In the day time, the process, ... is taking place in the fish, while the processes, ... and .... are occurring in the algae.

k. A balance is maintained in the sealed vessel by the two processes, ... and ....

l. The process, ... which can occur only in green plants, may be increased in rate by increasing the intensity of ....

6. A white rose and red rose were crossed. The hybrid was red. In order to produce only red roses from this hybrid, would you use seeds or cuttings? why?

7. Directions:

Read the description on the right hand side of the sheet below. Choose from the list of terms on the left hand side the one which best fits the description. Insert the *number* of the correct term in the blank space opposite the description. The same term number may be placed correctly upon more than one blank.

Description

1. annual rings
2. anther
3. bulliform   ....The movement of materials from a greater
4. cambium   to lesser concentration of the material.
5. cell division
6. chloroplast
7. chromoplast
8. chromosome
9. colenchyma
10. companion cell
11. cotyledon   ....Develops from the fertilized egg nucleus.
12. diffusion
13. digestion
14. elongation   ....A process in which energy is released.
15. embryo
16. flower

17. guard cell   ....Usually accompanies the sieve tube.
18. inflorescence
19. integument   ....A colorless plastid.
20. leaf
21. leucoplast   ....Region of cell division.
22. meiosis
23. meristem   ....Tissue in which foods are translocated.
24. parenchyma
25. pericycle   ....Region giving rise to secondary roots.
26. phloem
27. pistil   ....Cells, the walls of which are thickened at
28. respiration   the corners.
29. spikelet
30. transpiration   ....Bearer of determinants of hereditary char-
                            acters.

8. If you wished to study the effect of temperature on the rate of photo-
   synthesis, show how you would do so by designing an original
   experiment.

9. Near McGregor, Iowa, there is a burial mound built by the Indians.
   On this mound grows a white oak tree with a trunk diameter of two
   feet. How could you determine the minimum time elapsed since this
   mound was built?

10. Describe the process of cell differentiation from meristem to matur-
    ity, using a tracheal tube by way of illustration.

2.   Symposium: Erosion Prevention Capacity of Plant Cover

The emergency measures for the control of erosion have set in motion forces that call for direction in a field where only a limited amount of pertinent knowledge is available. We know our flora only in part. We know a little of its distribution and associations; and even less about the biology, growth response, and development as influenced by existing disturbed conditions. It is hoped that this symposium may stimulate wider interest in this field, especially among botanists and plant scientists.

CONTENTS

THE IMPORTANCE OF OUTDOOR PLANT STUDIES

B. Shimek

From the Department of Botany, State University of Iowa

Our outdoor water problem has so many inter-related and connected lines, angles and faces that the consideration or solution of any one of them involves all the others.

The need of a constant and sufficient, though not excessive supply of water for crop-plants, and the manifest influence of plants themselves on this supply through their effect on surface currents of air, have led to various attempts to regulate this moisture supply; and the mechanical effect of surface waters on erosion and during floods, has invited various measures for their control.

Though plants are so distinctly concerned with both cause and effect in connection with these problems, it is a curious fact that for their solution we have not summoned the student of plants, but rather the engineer and the meteorologist. The engineer has been called upon to direct water-conservation and water-distribution processes; he has determined the fate of trees along the highways; he has entirely dominated the field of artificial drainage and irrigation; and he has been the chief agent in working out erosion and soil conservation problems. Yet all these processes are primarily related to plants, either as the ultimate object of the procedure, or as a means towards the desired end. The meteorologist, on the other hand, has furnished the yard-stick in the form of the rain-gauge, for the measuring of conditions which determine the possibility of plant-growth. Yet neither one, in the course of his preparation or his professional experience, has had the opportunity to gain any knowledge of plant behavior, or the fundamental requirements for plant-growth.

It is a fact, though contrary to popular belief, that the plant ecologist is much more competent to judge of the character and control of the water-supply for these purposes than is the hydraulic engineer; and that the ecologist has developed a much more thorough understanding of the effect of precipitation and the loss through evaporation and run-off than either the engineer or the meteorologist. Yet, as a rule, he is not called upon to share in the solution of the problems involved.

Perhaps botanists themselves are in large part responsible for being thus neglected. They have so concentrated on the chase after chromosomes, the indoor detailed laboratory experimentation or demonstration, or the mere taxonomic study of plants, that they have neglected the divers combinations of causes and effects related to plants as displayed in the outdoor world. Even the economic botanist has been so anxious to find short-cuts to practical results, and has been so concerned with soil development, that he, too, has overlooked the full relationship of the factors involved; while too often the ecologist, who above all should be concerned, has been so occupied with the study of mere plant distribution, or with the development of a cumbersome and verbose terminology, that he, likewise, has often failed to appreciate the great scientific and economic significance

of the close investigation of cause and effect in the outdoor world.

The engineer and the meteorologist can render valuable service on the mechanical and physical side of the problems involving water-supply, drainage, irrigation and erosion, but the utlimate solution of these various problems in which plants are factors, must rest with the student of plants in all their relations—namely, the plant ecologist. For example, the engineer can decide if a place can be drained, but he is not prepared, either by training or experience, to determine whether it should be drained; the meteorologist can give valuable information on the distribution and amount of precipitation, but he is not in a position to judge fully of its ultimate behavior or disposition, distribution and diffusion after it has been precipitated—these are problems for the plant ecologist. It is high time that the botanists, and especially the plant ecologists, assert themselves in the economic field. A little has been done in connection with our western grazing lands, but this is only a fractional part of the responsibility which the plant ecologist should assume.

As noted, the fundamental facts and principles involved in most outdoor water-problems are more or less related to plants and to the conditions under which they exist. These problems may be divided into three great groups, the first including those related to atmospheric moisture, the second those concerned with surface and soil waters, and the third those which are connected with our surface bodies of water.

The first group involves relative humidity and its relation to evaporation and transpiration, and includes such practical problems as the proper development and use of windbreaks; the preservation of the sources of atmospheric moisture in swamps, ponds and lakes, now so often destroyed by drainage; the proper planting of trees and ornamental plants along highways; and the selection of suitable crops for different habitats.

The second group includes such problems as run-off and flood-control, soil-waters, evaporation and erosion. The problems of run-off and flood-control, as well as that of erosion, have been left largely to the engineer, who commonly employs cement and piling where plants would be just as effective and much less expensive; while the problems of soil-water and evaporation have received varied attention from meteorologist, engineer and geologist, with comparatively little emphasis on the value of plants as an important factor.

The third group embraces the problems related to our surface bodies of water, such as the conservation of fish, game and some fur-bearing animals, and other recreational features related to our waters.

These problems cannot be solved without due regard to the plant factors, nor does any one of them stand entirely apart from the others.

This symposium, for example, is concerned primarily with erosion problems, but these involve many factors related to other problems, the influence of which will be brought out, no doubt, in these discussions. Among them may be noted the amount and rate of run-off, so important in flood-control and the conservation of wild life; the amount and rate of evaporation which reduces the amount of run-off, but which is also all-important to plant growth; the amount of absorption of water by the soil, which also reduces the run-off, but is likewise of great importance to plant growth; the texture of the soil, so important in determining the rate of

erosion, but of equal importance because of its effect on the water-holding power of the soil, which, again, greatly affects plant-growth; and others to which reference may be made in the discussions.

That plants play an important part in checking or preventing erosion is obvious. They not only hold the soil by their roots, but they affect run-off by checking the velocity of surface currents, by creating a bed of leaf-mould which more readily absorbs water, by dashing much of the falling water into a fine spray which is more readily absorbed, and by retaining on their surfaces a considerable part of the precipitated water, thus enabling it to evaporate without reaching the soil. Some experimental work has been done to determine the effect of the various factors, to which no doubt references will be made during this session, but much more should be done to cover wider areas, since the factors involved do not operate in equal degree under the divers and varying conditions of our broad land. Emphasis should be placed also upon the effect of erosion on our soils, and on our streams and lakes, which suffer from silting.

The various erosion problems present three rather distinct phases: Erosion of cultivated lands where the disturbance is more or less continuous; erosion of the bluffs and rougher areas which have been stripped of their forests or deprived of their prairie covering; and erosion on bottom-lands subject to overflow. Each of these types presents special problems, though all are closely related.

In all cases, however, plants play a very important role, and it is gratifying that this program will manifestly emphasize that fact.

THE EFFECT OF PLANT COVER ON SOIL AND WATER LOSSES

R. E. UHLAND

From the Soil Erosion Service, United States Department of Interior

It has been shown that the removal of forest growth, grass and shrubs and the breaking of the ground surface by cultivation, the trampling of livestock, etc., accentuate erosion to a degree far beyond that taking place under natural conditions, especially on those soils that are peculiarly susceptible to rainwash. It has long been realized that the speeding up of the washing varies greatly from place to place according to soil character, climatic conditions, vegetative cover, degree of slope, disturbance of the ground surface and depletion of the absorptive organic matter in the soil under continuous clean cultivation.

While little is known about the actual rate of soil formation we do know that it is extremely slow. Mr. H. H. Bennett has often stated that it requires more than 400 years to produce a single inch of surface soil and he is undoubtedly very conservative in his estimate. We are certain that the formation is so slow that it is not keeping pace with the rapid removal that is under way as the result of artificial disturbance of the vegetative cover and ground equilibrium through chiefly the instrumentality of man and his domestic animals.

The removal of the natural cover of vegetation, which for Iowa and Missouri was chiefly prairie grasses, and the disruption of the normal porosity of the soil with plows and domestic animals caused accelerated runoff of rainwater and consequent erosion. Despite the fact that these forces altered the ground surface so greatly, the fundamental processes involved with these man-induced changes of natural soil conditions were not recognized or studied in any systematic manner until within the past fifteen years.

You have all perhaps read accounts of recent surveys which show that more than 75 per cent of the crop-producing and grazing areas of the United States are sloping enough to favor more or less serious soil erosion. Investigations show that more than 35 million acres of formerly cultivated land have been essentially ruined by erosion and that an additional area of about 125 million acres, still largely in cultivation, has lost all or most of the top soil, with another 100 million acres of crop land heading in this direction.

It was for the purpose of actually studying the many variables and modifying factors involved in soil erosion in a scientific manner that there was set up under the Department of Agriculture several years ago an experimental erosion control program with experiment stations in different sections of the country. Since soil type alone introduces an almost endless variety of conditions that appreciably and even profoundly affect the rates of absorption and therefore of runoff and soil denudation, it was necessary to locate these experiment stations on different soil types. These Soil Erosion Stations were established in cooperation with the Experiment Stations of the states in which they were located. Mr. H. H. Bennett,
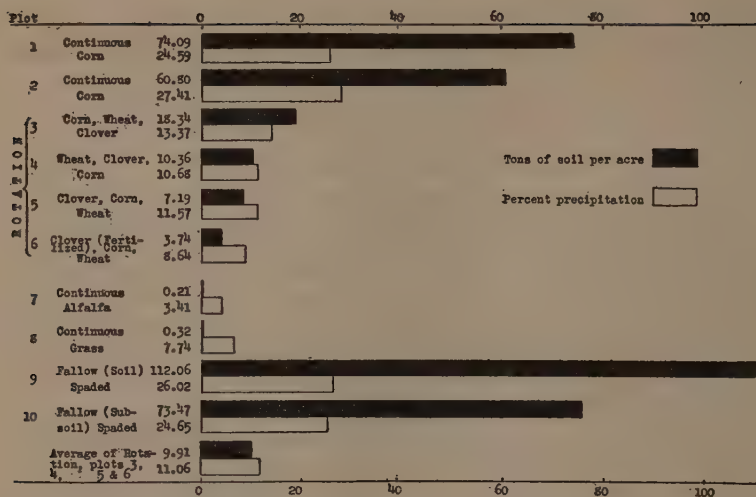
Plot		Tons of soil per acre	Percent precipitation
1	Continuous Corn	74.09	24.59
2	Continuous Corn	60.80	27.41
3	Corn, Wheat, Clover	15.34	13.37
4	Wheat, Clover, Corn	10.36	10.68
5	Clover, Corn, Wheat	7.19	11.57
6	Clover (Fertilized), Corn, Wheat	3.74	8.64
7	Continuous Alfalfa	0.21	3.41
8	Continuous Grass	0.32	7.74
9	Fallow (Soil) Spaded	112.06	26.02
10	Fallow (Sub-soil) Spaded	73.47	24.65
	Average of Rotation, plots 3, 4, 5 & 6	9.91	11.06

Shelby silt-loam, slope 8 per cent, mean precipitation, 33.54 inches; Plot 1, 6 x 145.7 ft.; plots 2 to 10, inclusive, 6 x 72.85 ft.

GRAPH 1. Average annual soil and water losses. Source: Erosion Experiment Station, Bethany, Missouri. Period: Years 1931 to 1933, inclusive.

Plot		Tons of soil per acre	Per cent precipitation
1	Corn Ground, bare not plowed	53.62	66.6
2	Corn Ground, bare not plowed	45.55	68.6
3	Clover Stubble (Corn to follow)	0.23	29.6
4	Wheat (Blades 2 to 3 inches long)	11.31	72.2
5	Clover, seeded Spring 1933, unfertilized	1.55	43.0
6	Clover, seeded Spring 1933, fertilized	0.465	39.0
	AVERAGE, plots 3, 4, 5, and 6	3.40	45.75
7	Alfalfa (just beginning to grow)	0.30	40.5
8	Bluegrass (just beginning to grow)	0.085	39.2
9	Fallow, fall plowed	28.49	52.7
10	Fallow on subsoil, fall plowed	29.61	74.5
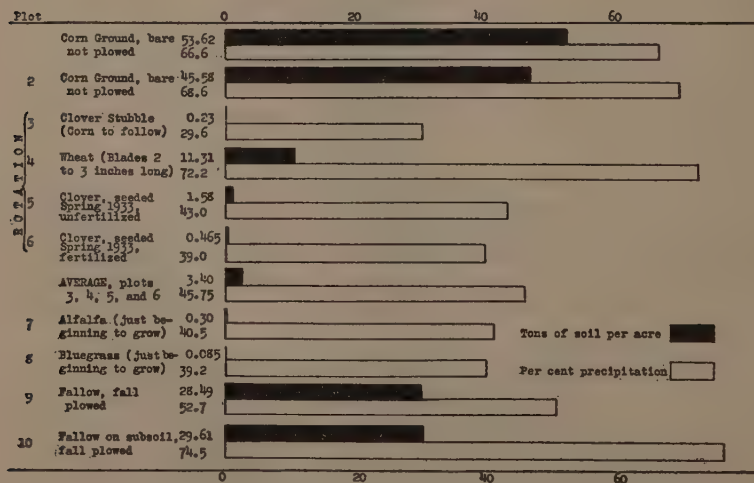
Shelby silt-loam, slope 8 per cent; 3.30 inches fell at rate of 2.36 inches per hour. Plot 1, 6 x 145.7 ft.; plots 2 to 10, inclusive, 6 x 72.85 ft.

GRAPH 2. Results of one rain of 3.71 inches, April 3, 1934. Source: Soil Erosion Station, Bethany, Missouri.

Director of the Soil Erosion Service, planned and developed the program of investigation of the Bureau of Chemistry and Soils while the engineering phases of erosion control were directed by the Bureau of Agricultural Engineering.

Most of you are familiar with the fact that two of those soil erosion experiment stations are located close at hand. One of these stations is located at Bethany, Missouri, on the Shelby loam and Shelby silt loam soils, and the other at Clarinda, Iowa, on the Marshall silt loam. The station at Bethany, consisting of 220 acres, was started in the spring of 1930, and the Clarinda station, consisting of 200 acres, was started in the spring of 1932. With the extreme variations of seasons and the amount and intensity of rainfall from year to year it is evident that considerable time must elapse before absolutely accurate conclusions relative to erosion may be drawn. However, the results to date with regard to the effect of plant cover on soil and water losses are highly significant. Since the writer was directly responsible for securing the results at the Bethany station, most of this discussion will be confined to that station with introduction of sufficient data from the several other stations of the United States to show clearly the important part played by vegetation in controlling both soil and water losses. That the soil type plays an important part in regulating the rate of water percolation or infiltration into the soil is shown by graphs 4 and 5. The infiltration rate for the Marshall loam was seven times greater than for the Shelby loam.

The results secured at Bethany covering a period of three years (1931-33) inclusive, are illustrated in graph 1. These measurements were made on an 8 per cent slope and the mean precipitation for the three year period was 33.54 inches. The length of all but one of these plots is 73 feet and the length of the long plot is 146 feet. It will be noted that even on this short slope, which represents roughly the cross-section between terraces, the average annual soil loss has exceeded 60 tons per acre, along with 27 per cent of total precipitation, where corn is grown continuously. The loss from exactly the same kind of soil, having identical slope and receiving the same rainfall, but devoted to thick-growing crops, was surprisingly low. Under alfalfa there has been a loss of only .2 ton of soil per acre and about eight per cent of the precipitation. These results show alfalfa to be about three hundred times, and the grass about two hundred times more effective than corn in holding soil and thus preventing erosion. Alfalfa proved nine times as efficient in reducing runoff as corn, and grass was three times as effective. For the same period, fallow land kept free of vegetation lost an average of 112 tons of soil or more than five hundred times as much from the plot cropped to alfalfa. From areas cropped under a three-year rotation of corn, wheat, clover and timothy the loss of soil where neither fertilizer nor lime was used was at the rate of little less than 12 tons per acre, as compard with only 3.74 tons per acre where lime and fertilizer were applied and the same rotation used.

The above results show that lime and fertilizer applied once in the rotation may greatly reduce erosion. This fact was very forcefully demonstrated in 1932 when land in corn following clover that had formerly been limed and fertilized at wheat seeding time lost soil at the rate of 9.8 tons per acre, as compared with a loss of 19.6 tons per acre from adjoining land, which was not limed or fertilized, but which had the same cropping

		Tons of soil per acre	Per cent precipitation
1	Fallow, plowed 4 inches	41.6	31.0
2	Fallow, plowed 8 inches	41.2	31.3
3	Continuous Wheat	10.1	24.0
4	Continuous Corn	19.7	30.3
5	ROTATION, corn, wheat, clover	2.7	14.4
6	Continuous Bluegrass	0.3	12.5
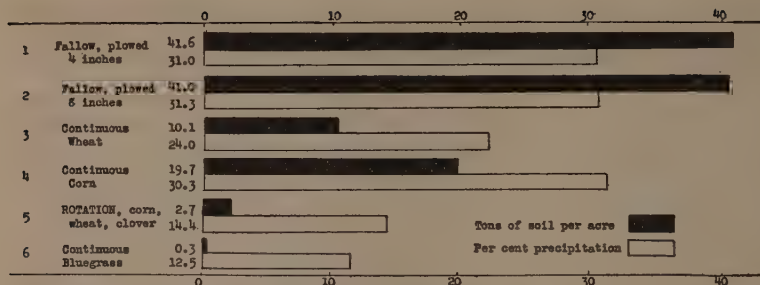
Shelby loam, slope 3.68 per cent; mean precipitation, 40.37 inches. Plots 1 to 6, in-
clusive, 6 x 90.75 ft.

GRAPH 3. Average annual soil and water losses, 1918-31, inclusive. Source: Agricul-
tural Experiment Station, Columbia, Missouri.
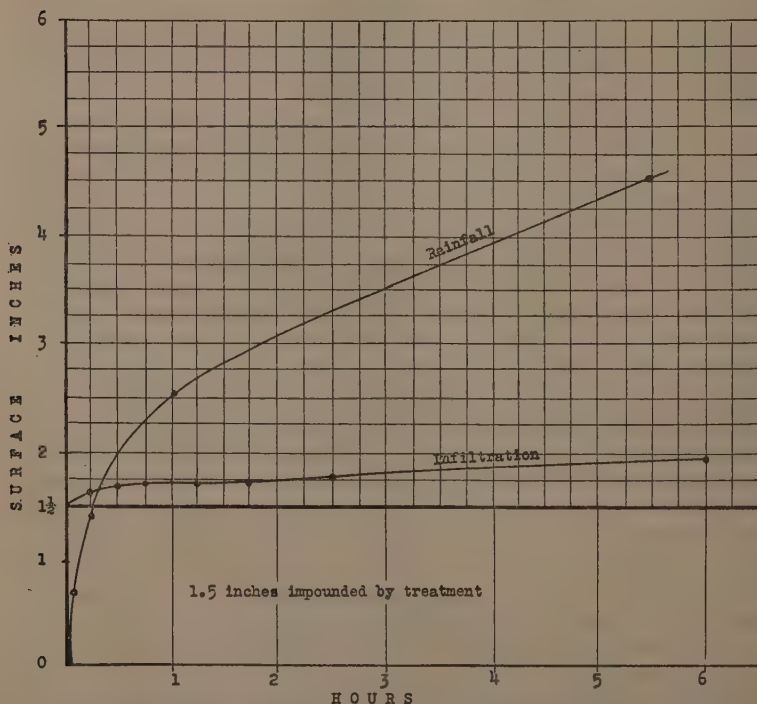
Infiltration rates determined on soil of normal structure, moist, sod removed.

GRAPH 4. Protection provided against runoff for rain of rare intensity and duration,
Shelby silt loam. Source: Soil Erosion Station, Clarinda, Iowa.
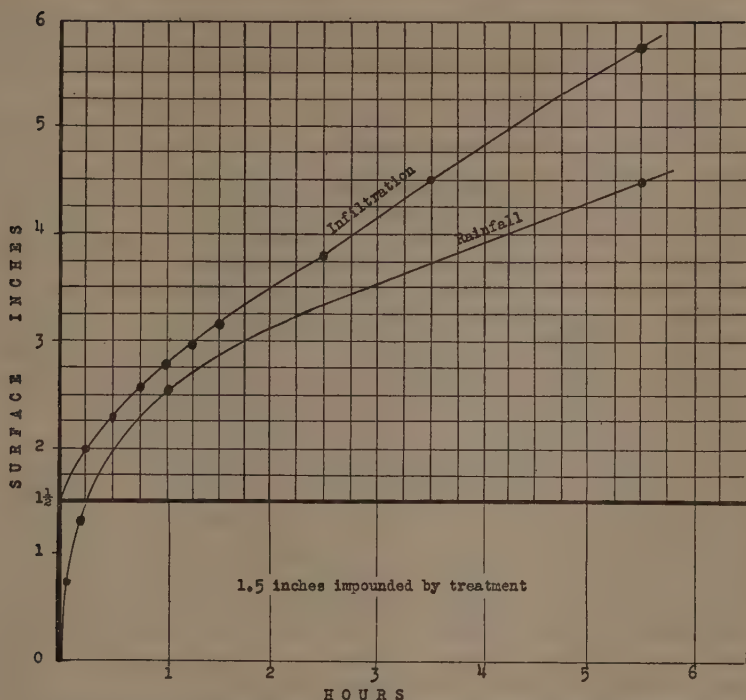
Infiltration rates determined on soil of normal structure, moist, sod removed.

GRAPH 5. Protection provided against runoff for rain of rare intensity and duration, Marshall silt loam. Source: Soil Erosion Station, Clarinda, Iowa.

system. This same year land in corn following corn lost soil at the rate of 48.6 tons per acre, which was more than five times the loss from fertilized land on which a good cropping system was followed. This indicates that there is a marked residual effect of both the preceding crop and fertilizer treatment. It shows also that there is a marked difference in the soil loss from land in the same crop, especially where the crop is poor. This latter point was demonstrated on Experiment 2 in 1932 and again in 1933. In 1932 on another experiment and on badly eroded land seeded to oats and red clover the untreated area lost 9.75 per cent of the rainfall, while no runoff was recorded for the treated plot. For 1933 the fertilized plot yielded a crop of good clover hay amounting to slightly more than one and one-half tons per acre, while the untreated plot yielded only 1,400 pounds of crab grass and a little clover. While little soil was lost from either plot, the treated plots lost only 20 per cent as much water as was lost from the untreated plot. These results further demonstrate that not only is there a marked difference in the soil and water losses from different kinds of crops, but also from the same crop, where a good crop is com-

pared with a poor crop. The results show that the loss from a good crop, whether it be a cultivated crop or a close growing sod crop, is much less than from a poor crop, which demonstrates an added need for keeping up the fertility and productivity of soil.

It is of course obvious that these experiments must run for a long period of years so all types of rains, together with soil and crop conditions, may be encountered, before too many conclusions may be drawn. This is well demonstrated by graph 2, which gives the soil and water losses caused by a single rain totaling 3.71 inches, which fell April 3, 1934. This rain was quite intense (3.03 inches fell at an average of 2.36 inches an hour). From plot 1 in continuous corn on the long slope the soil loss for this single rain exceeded 53 tons per acre, which was a greater loss than occurred as a result of the entire annual precipitation on this same plot for 1932. The soil loss from plot 2, which was one-half the length of plot 1, and which was also in corn, was about 46 tons per acre. More than two-thirds of the rainfall was lost from each of these plots. The soil loss from plot 3, which was in clover, was .23 ton per acre and the water loss was 29.6 per cent, or only 44 per cent as great as the water lost from corn land.

Graph 3 shows the average annual soil and water losses, 1918-31, inclusive, secured on Shelby loam with a slope of 3.68 per cent at Columbia, Missouri. The average annual precipitation was 40.37 inches. It will be noted that the soil loss from fallow land was at the rate of 41 tons per acre, from corn it was 19.7 tons per acre, from continuous wheat 10.1 tons per acre, for crops in rotation the loss was but 2.7 tons per acre, while land in continuous bluegrass lost only .3 ton of soil per acre per year.

Table 1 shows that when grass occupies the land the degree of slope exerts little effect on the soil loss. On the other hand, when corn is grown on an 8 per cent slope the average annual loss (4 year average) was 61 tons; whereas, on a 3.7 per cent slope the soil loss was only 20 tons per acre (14 year average). In other words, doubling the slope on Shelby silt loam increased the soil loss three times where corn was grown, while no effect was indicated when these two slopes were in grass.

Table 2 shows the results secured at Tyler, Texas, and it is noted here again that the soil loss from very steep slopes (16.5 per cent) lost scarcely no soil when in grass, while the loss ran as high as 35 tons per acre when in cotton.

The relative effectiveness of forest cover and grass on erosion losses is indicated in table 3. This data was secured by the Bureau of Chemistry and Soils at Tyler, Texas, and Guthrie, Oklahoma. The results show that forest, as well as good stands of grass, gives practically complete protection from erosion on these very extensive and important soil types. Water losses were extremely small as compared to those from burned-over land. Results similar to these were also secured at Statesville, North Carolina.

The relative soil and water losses recorded for several different soil erosion experiment stations, covering a period of from four to fourteen years, are shown in table 4. These results show very conclusively the important part that the plant cover plays. In every case the losses from close and dense growing vegetation were very low and a good crop rotation was quite effective in materially reducing both soil and water losses.

TABLE 1. *Loss of soil and water from Shelby soil occupying different slopes*

Location	Soil	Slope	Soil-loss		Water-loss	
			Corn	Grass	Corn	Grass
		Pc't'g	tons per acre		Pc't'g precipitation	
Bethany, Missouri (4-year average)	Shelby silt-loam (nearly a loam)	8.0	61	0.3	27	8
Columbia, Missouri (14-year average)	Shelby loam	3.7	20	0.3	30	13

TABLE 2. *Loss of soil and water from Kirvin and Nacogdoches soils, East Texas*

Location	Soil	Slope	Soil-loss		Water-loss	
			Cotton	Grass	Cotton	Grass
	fine sandy loam	Pc't'g	tons per acre		Pc't'g precipitation	
Erosion-station near Tyler, Texas	Kirvin	8.75	19	0.2	20	1.5
Erosion-station near Tyler, Texas	Kirvin	16.50	35	0.0	13	0.7
Erosion-station near Tyler, Texas	Nacogdoches	10.00	6	0.02	15	1.4

TABLE 3. *Effect of forest on erosion and runoff, as compared with grass Tyler, Texas, and Guthrie, Oklahoma*

Soil	Mean pre-cipita-tion	Slope	Cover	Soil-loss	Water-loss
fine sandy loam	inches	Pc't'g		tons per acre	Pc't'g of pre-cipitation
Kirvin	44.4	12-1/2	Forest	0.01	0.8
Kirvin	44.4	12-1/2	Forest, litter burned	0.19	2.6
Kirvin	42.3	8-3/4	Bermuda grass	0.21	1.5
Kirvin	48.8	16-1/2	Bermuda grass	0.00	0.7
Vernon	33.5	5-1/5	Forest	0.017	0.13
Vernon	33.5	5-1/5	Forest, litter burned	0.22	5.06
Vernon	32.9	7-7/10	Bermuda grass	0.04	1.5

TABLE 4. *Comparison of soil- and water-losses under crop rotations on eight important soils*

Location	Soil	Slope	Rotation	Mean precip.	Soil-loss	Water-loss
		Pc't'g		inches	tons per acre	Pc't'g of precipitation
Bethany, Missouri.	Shelby silt-loam	8	Corn, wheat, clover	34	10	11
Columbia, Missouri	Shelby loam	3.7	Corn, wheat, clover	40	10	24
Hays, Kansas	Colby silt clay-loam	5	Wheat, kafir, fallow	22	10	16
Guthrie, Oklahoma	Vernon fine sandy loam	7.7	Wheat, sweet clover, cotton	33	6	12
Temple, Texas	Houston black clay	4	Cotton, corn, oats	27	6	5
Tyler, Texas	Kirvin fine sandy loam	8.75	Cotton, corn, lespedeza	42	16	18
LaCrosse, Wisconsin	Clinton silt clay-loam	16	Barley, corn, clover	29	21	13
Statesville, North Carolina.	Cecil sandy clay-loam	10	Corn, wheat, lespedeza, cotton	43	7	10

CERTAIN ASPECTS OF THE ROLE OF VEGETATION IN EROSION CONTROL

W. C. LOWDERMILK

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The nature of interrelations between vegetation and soil erosion has been disclosed by experimental studies, which have been in progress for nearly two decades. Duley and Miller (1923) began in 1917 a series of studies on erosion and run-off as affected by different crops and methods of cultivation, which have had a profound influence on quantitative studies of this nature. The writer adopted the plot method in 1923 in China (1926), and with subsequent studies in California (1930) discovered that the accumulation of a layer of ground litter under natural forest vegetation serves to exercise an influence of far reaching significance in controlling storm run-off and soil erosion, and likewise in problems of land-use planning. Bennett inaugurated in 1928 a national program, prepared in 1926, of research in soil and water conservation under the Bureau of Chemistry and Soils, in the establishment of ten soil erosion stations throughout the United States (1933). These stations are supplying basic information in limits of sustained land use. Studies by the Branch of Research of the United States Forest Service by Sampson (1918), Bates (1928), Auten (1932), Stickel (1931), Cooperrider (1931), Forsling (1931), and others are supplying quantitative data which must furnish the information required for planning the sustained use of land resources, in the conservation of its soil and water supply.

The baring of a soil to the full effects of dashing rain and blasts of wind against which it was formerly protected by a closed mantle of vegetation has been found in these experimental studies to increase the surficial run-off from bared surfaces and rates of soil wash to significant and important amounts. This finding varies with type of soil slope, type of precipitation, and type of land use. On forest lands removal of vegetative coverage by fire increased surficial run-off up to more than forty times that from unburned surfaces during a storm of over eleven inches (figure 1). Forsling likewise showed (1931) the tremendous increase in run-off following removal of natural grass coverage by over-grazing. Cultivation, moreover, is the principal means of accelerating soil erosion as may be seen from results of studies at the Soil Erosion stations by the Bureau of Chemistry and Soils, under the direction of Bennett and Allison (figures 2 to 7).

It is interesting to compare experimental results at Hays, Kansas, at Guthrie, Oklahoma, and Statesville, North Carolina, between erosion and run-off, from the natural forest plots and the cultivated plots.

These informative graphs also indicate the effect of various types of crops, rotations in crops, and plowing in of cover crops. From these data it becomes apparent immediately that sustained use of agricultural soils is subject to certain definite limitations. First: wherever sloping lands are bared they are subject to soil losses at cataclysmic rates in
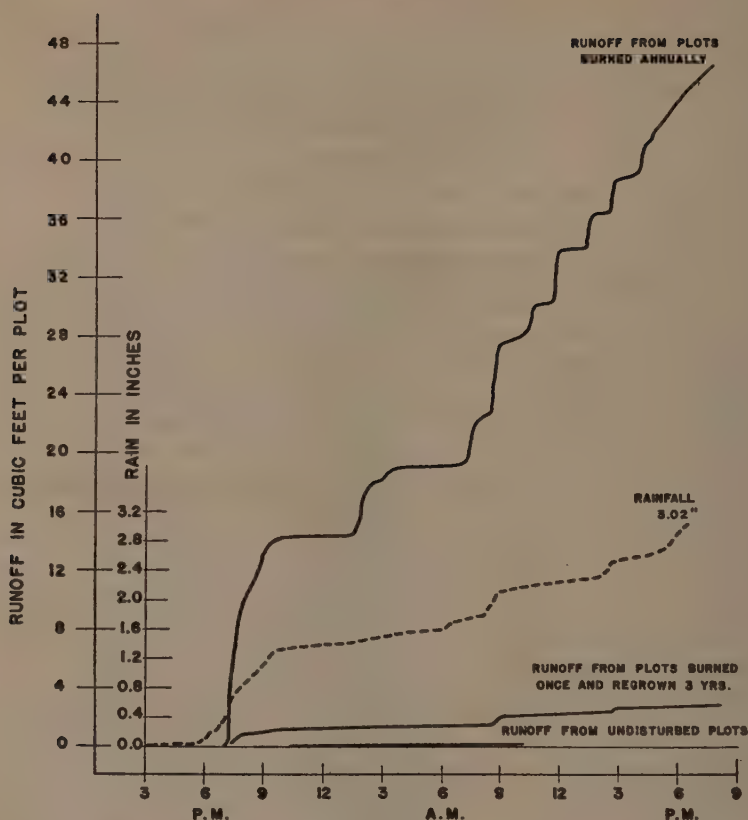
Fig. 1. Source: California Forest Experiment Station, North Fork, Calif. Period, Dec. 31 to Jan. 1, 1934. Sierra brush type, slope 32 per cent; area of plots 1/40 of an acre.

comparison with those from soils clothed with their natural coverage of vegetation or with grass. Since the rate of loss under undisturbed vegetation may be accepted as the rate of a geologic norm of erosion for that condition, it is certain that soil erosion from the bared sloping surfaces of these experiments is in excess of this norm: It is surely in excess of soil formation. For example: The experiments at Hays, Kansas, disclose the interesting fact that clean cropped land has lost under a mean annual rainfal of 22.18 inches, an average annual soil loss from native grass of 0.09 tons per acre, and surficial run-off equivalent to 0.64 inches of rain; whereas, the losses from a field rotated to wheat, kaffir corn and fallow were 15.79 tons of soil per acre and 16.34 per cent of the rainfall. This means that under natural grass it would require more than 16,000 years to wash away the same depth of soil under this type

of cultivation and cropping. Soil formation can be expected to proceed as rapidly as soil removal in the first instance, and much less rapidly in the second.

The method of experimentation which gave rise to these results scarcely needs description since it has been reported in former papers by Duley and Miller (1923), Bennett (1933) and the writer (1934). Essentially sloping plots of 1/100 acre to 1/40 acre are bordered and fitted with devices at the lower end to catch and measure surficial run-off and
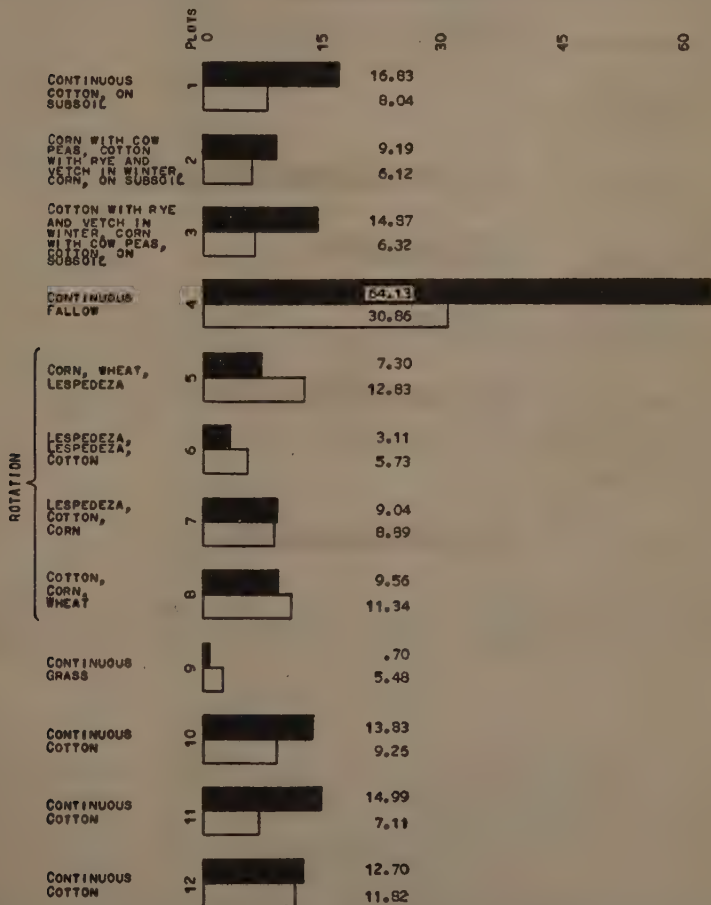
Fig. 2. Average annual soil and water losses. Source: Piedmont Erosion Station, Statesville, N. C. Cecil sandy clay loam, slope 10 per cent, mean precipitation 42.9 inches. Plots 1 to 10, inclusive, 6 x 72.6 ft; plot 11, 6 x 145.2 ft; plot 12, 6 x 36.3 ft.
■ tons of soil per acre
□ percentage of precipitation
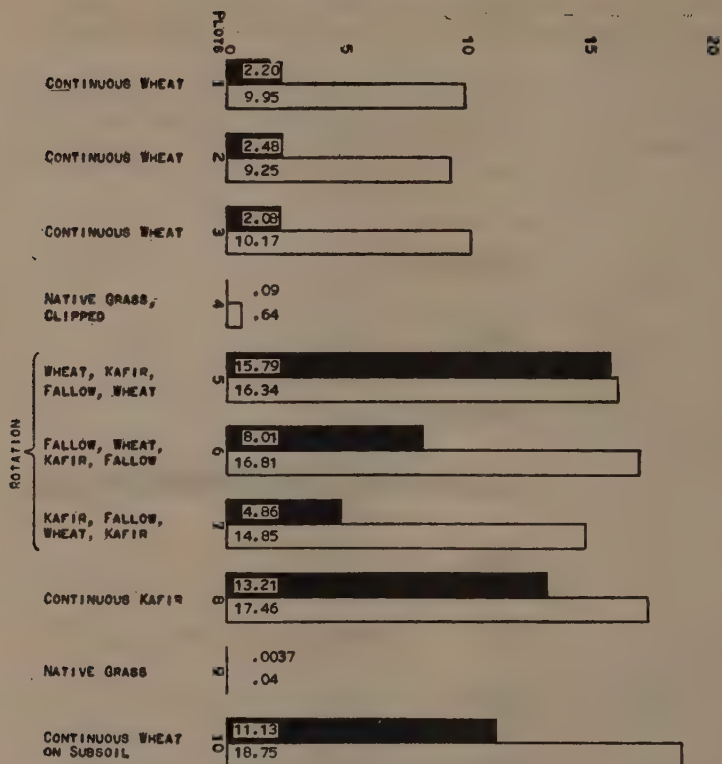
Fig. 3. Average annual soil and water losses. Source: Western Kansas Plains Station, Hays, Kansas. Period: Years 1030 to 1933, inclusive. Colby silty clay loam, 5 per cent slope, mean precipitation, 22.18 inches. Plot 1, 6 x 36.3 ft.; plot 2, 6 x 145.2 ft.; others, 6 x 72.6 ft.

■ tons of soil per acre
□ percentage of precipitation

eroded material. Plots for comparative studies are similar in all respects except the surface conditions, which are made to represent natural forest cover, various methods of cropping, and fallowing. The results from a series of these installations are shown in graphs in figures 1 to 7. The experimental installation at Guthrie, Oklahoma, has yielded likewise a number of interesting and significant comparisons. Average results from 1930-33 under an average of 33.49 inches of rain per annum, were from plots in virgin woods, erosion, 0.017 tons per acre; run-off, 0.13 per cent of rainfall; and from plots cultivated continuously to cotton, erosion 39.62 tons per acre; and run-off 14.04 per cent of rainfall (figure

6). It is apparent from these results that a complete coverage of vegetation in grass or forest maintains the integrity of the soil in the most complete manner.
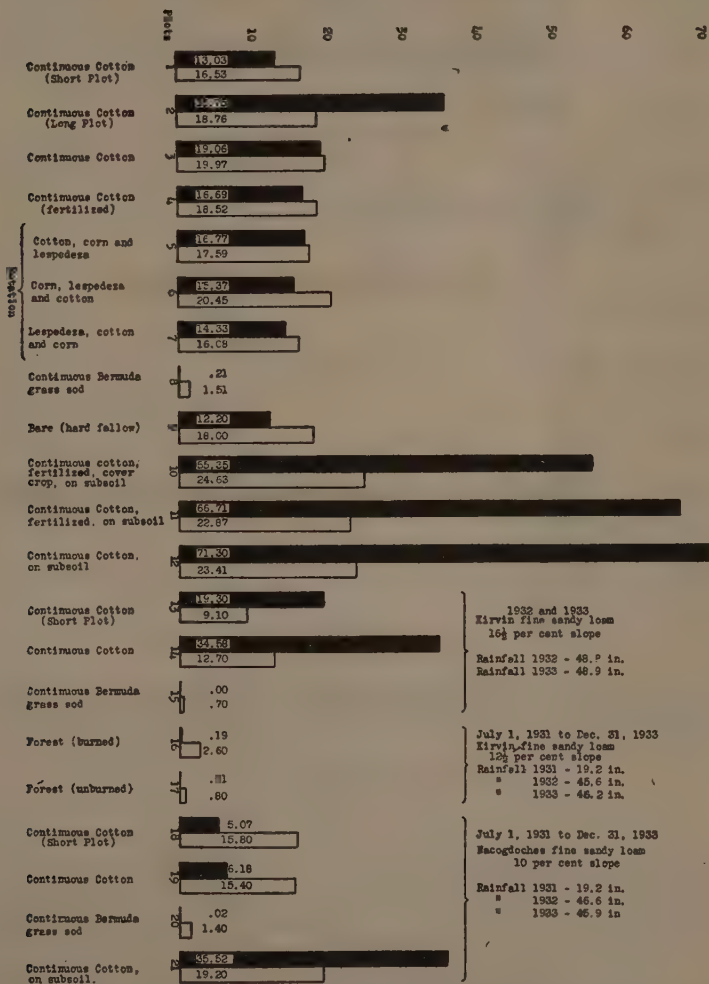
Fig. 4. Average annual soil and water losses. Source: Erosion Station, Tyler, Texas. Period: Years 1931 to 1933, inclusive. Kirvin fine sandy loam, slope 8.75 per cent; mean precipitation, 42.31 in. Plots 1, 13, 18, 6 x 36.5 ft.; plot 2, 6 x 145.8 ft.; others, 6 x 72.9 ft.

■ tons of soil per acre
☐ percentage of precipitation
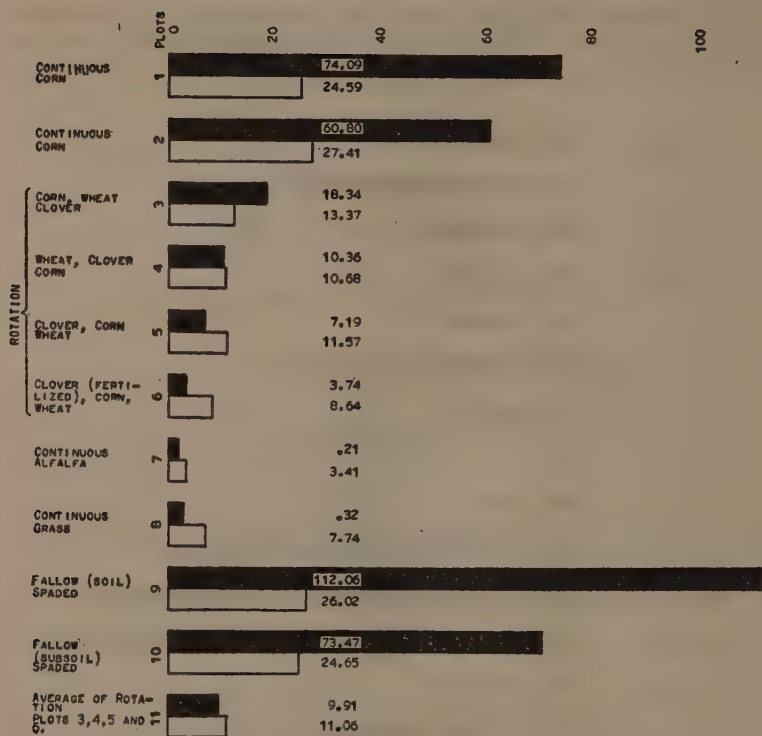
Fig. 5. Average annual soil and water losses. Source: Erosion Experiment Station, Bethany, Missouri. Period: Years 1931 to 1933, inclusive. Shelby silt loam, slope 8 per cent; mean precipitation, 33.54 inches. Plot 1, 6 x 145.7 ft.; plots 2 to 10, inclusive, 6 x 72.85 ft.

■ tons of soil per acre
□ percentage of precipitation

On the other hand, it is necessary to clear land for cultivation to food and textile crops. The problem then becomes one of discovering what lands may safely be cleared for cultivation, and what methods of use are required to maintain their productivity in safeguarding them from soil wastage by accelerated erosion. The solution of the problem of sustained land use under cultivation involves full recognition of all studies of this nature. Either cultivation must be restricted to level bottom lands, or sloping lands must be level terraced, or special methods and devices must be applied to sloping lands which must not exceed specific gradients.

Until American agriculture can afford to level terrace its sloping lands, as did the Incas, certain limitations and rededication to use seem to be necessary, if soil wastage is to be curtailed and water conservation is to be effected. Zoning in allowable uses and methods of cropping is

indicated. Such is the task of the land-use planner under the present status of economic conditions.

In the irrigated west the problem possesses different features but the same portents. In regions of seasonal and limited rainfall impoundage of waters from the more copiously watered mountains is necessary to the important development of irrigation and to municipalities. Acceleration of erosion and flash flood run-off on watersheds draining into these stor-
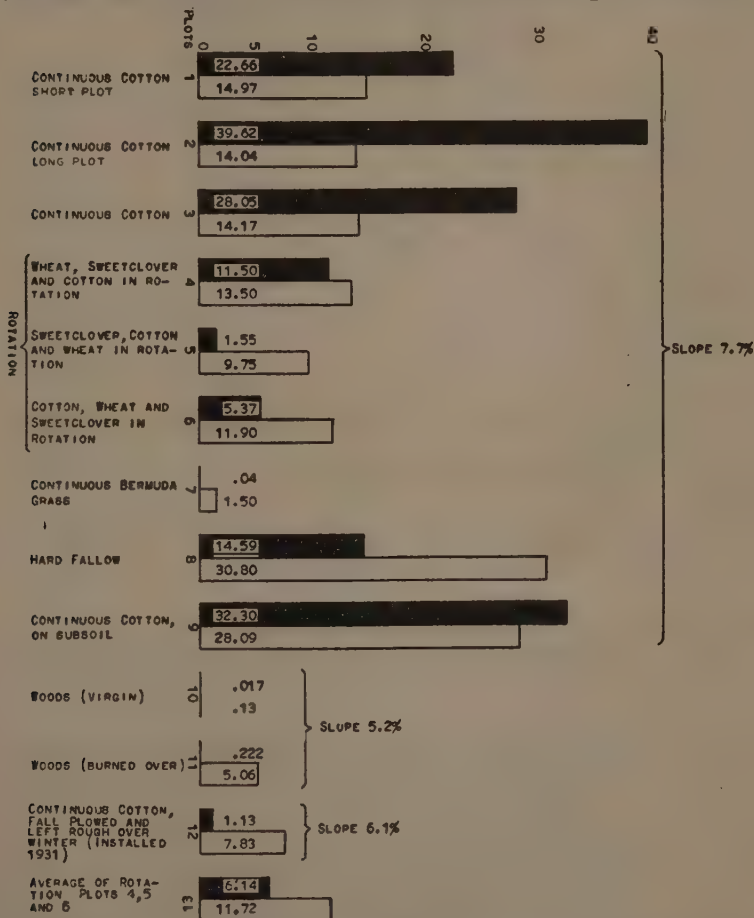
Fig. 6.   Average annual soil and water losses. Source: Red Plains Erosion Station, Guthrie, Oklahoma. Period: Years 1930 to 1933, inclusive. Vernon fine sandy loam; mean precipitation: Plots 1 to 9, inclusive, 32.92 in.; plots 10 to 12, inclusive, 33.49 in. Plot 1, 6 x 36.3 ft.; plot 2, 6 x 145.2 ft.; others, 6 x 72.6 ft.
■ tons of soil per acre
□ percentage of precipitation
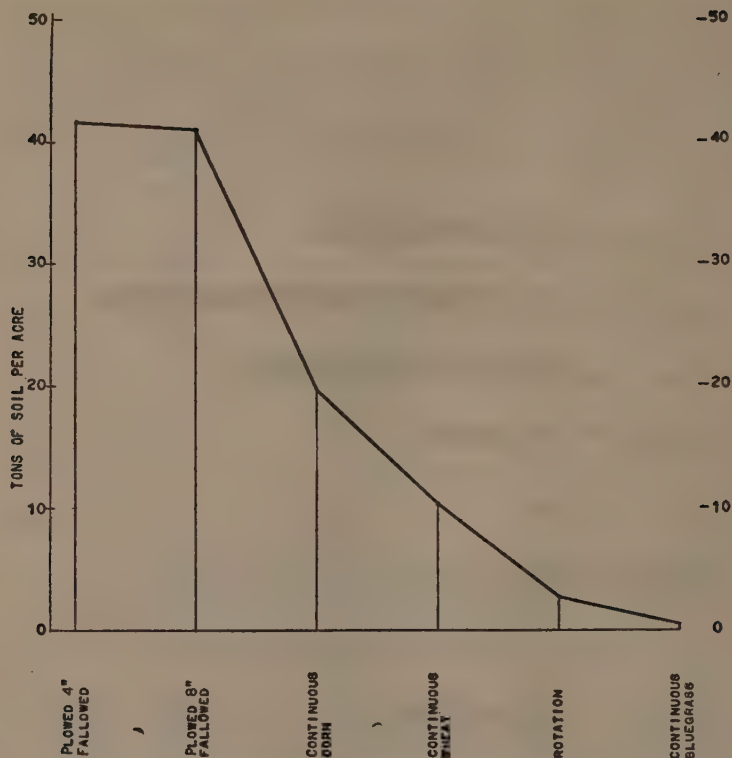
Fig. 7. Average annual soil loss by erosion from six plto treatments for fourteen-year
    period, 1918 to 1931, inclusive. Source: Agricultural Experiment Station, Colum-
    bia, Missouri. Shelby loam, slope 3.68 per cent; mean annual precipitation, 40.37 in.

age reservoirs becomes a direct menace to the investments of hundreds
of millions of dollars and to the social security of millions of people. The
loss of surface control of natural vegetation by destructive lumbering
and fire in the forests and by overgrazing in the grass lands has set in
motion headlong processes of erosion of mountain slopes and the cutting
out of alluvial filled upland valleys. The Elephant Butte irrigation project
may serve as a portentous example of this menace. The Elephant Butte
Reservoir of New Mexico, with an original storage capacity of 2,638,860
acre feet, supplies one of the largest irrigation projects of the West and
is silting up at a disquieting rate. It was originally forecast to have a life
of 233 years on the basis of silt studies of the Rio Grande from 1897-1912.
After the completion of the Elephant Butte Dam in 1915, silt surveys
have been regularly made in the reservoir. From 1915 to 1925 the aver-

age annual rate of silting was estimated from silt surveys at 20,000 acre feet, forecasting a life of 132 years; from 1926 to 1929, inclusive, 21,943 acre feet forecasting a life of 110 years. Erosion of alluvial valleys above the reservoir is increasing yearly. But still more ominous is the fact that within approximately 60 years, the storage capacity of this reservoir would equal the annual draft of water for the irrigated land. During dry years the lands will suffer a shortage of water and from that time on irrigation under the Elephant Butte Reservoir will be a precarious enterprise.

Underground reservoirs of extensive detrital fills play a more important rôle than surface reservoirs in certain sections of the west. Heavy pumpage from these basins has progressively lowered water tables to critical depths. Replenishment of these basins is the most urgent need of these regions. Such replenishment is achieved by sinking of run-off waters from mountain watersheds. Successful spreading and sinking of run-off waters is influenced in important degree by their clarity; muddy waters seal up spreading grounds and flow away as waste to the ocean. On the other hand, so critical has become the supply that the question has been raised by some, knowing that the brush and chaparral forest cover transpires moisture, whether it would not be advisable to destroy such non-commercial vegetation by fire so as to recover in streamflow a portion of the transpiration loss. This is a complex question, which requires carefully planned studies to answer authoritatively, and at the same time to place the land-use planning for the watersheds upon a scientific basis (Lowdermilk, 1934).

From such studies which are disclosing the rates of acceleration of physiographic processes by baring of soils and the water usage by vegetation, there may be drawn significant conclusions which must govern the planning of land-use. Level lands are the safest site of sustained agriculture. If cultivation must extend to sloping lands certain limitations are required. Likewise in safeguarding water supply for municipal and irrigation use, the management of extensive mountain water-yielding drainage areas must rest upon definite limitations.

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MOSSES AND SOIL EROSION

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Hitherto the mosses have been considered a difficult group for the collector, because of their small size. With modern helps, and with the development of local lists and keys, the recognition of moss species should not be more difficult than the recognition of grasses, and not so difficult as the sedges. By "modern helps" I mean first of all the compound microscope as an ordinary and familiar tool, the advent of which conditions we are here celebrating. Quite as indispensable is the compound binocular dissecting microscope. The lively text and lifelike figures in Dr. Grout's "Mosses with Hand Lens and Microscope" (which is the source of the names used in this paper) place a knowledge of mosses within the reach of any patient and observing person in the northeastern states. Grout's new "Moss Flora of North America" is doing as much for students everywhere on this continent.

If erosion does not begin, it will not proceed. Only on this basis can such small plants as the mosses be considered as agents in the arrest of soil erosion. Rarely reaching more than one-half inch into the soil, they can do little to withstand undercutting. And only rarely can they obstruct the work of permanent streams or of strong, rapidly moving temporary runnels. That they do function very extensively in checking primary sheet erosion is perfectly obvious. In this respect mosses act in the same way as leaf mold or duff: the impact of rain drops is caught, much of the water is held where it falls, and the run-off is reduced and retarded. In fact, in a paragraph by Borthwick (1934) on the effect of forests upon water, every retarding influence that he mentions is distinctly aided by mosses.

In order to determine the water-holding capacity of moss, we have weighed a clump of *Anomodon attenuatus* from Monticello, Iowa, after drying on the laboratory table for three weeks in October. The weight was 8 gm. It was then dipped in distilled water at room temperature and held submerged for seven minutes. With excess water shaken out it weighed 26 gm. It was then pressed between towels, when it weighed 23 gm. Thus a dry clump of *Anomodon attenuatus,* Iowa grown, can absorb two and one-half times its weight of water in seven minutes. It should be noted that this moss is extremely abundant on shaded banks in Iowa, especially on weathered limestone. Also, the Anomodons absorb water with astonishing rapidity; a dry and shrivelled fragment thrown into a dish of water spreads out completely in 40 seconds.

A clump of *Bartramia pomiformis* weighing 3 gm. dry weighed 19 gm. after one hour in distilled water. This represents absorption of five and one-third times the dry weight of the moss. Bartramia often covers many square feet of shaded banks in Iowa woods. It absorbs water relatively slowly.

Fernow is quoted as saying (Borthwick, 1934), "The forest litter, the moss-covered leaf-strewn ground, is capable of absorbing water at

the rate of 40,000,000 to 50,000,000 cubic feet per square mile in ten minutes, water whose progress is delayed by some twelve to fifteen hours after the first effects of a heavy freshet have passed."

The relation of mosses to run-off is two fold. First, there is no turbidity in the run-off from moss-covered ground. How often we notice crystal-clear water dripping from an overhanging blanket of moss! Secondly, turbid water flowing into a bed of mosses deposits much of its sediment there. On a steep gravel slide at Cold Spring Harbor, Long Island, a heavy rain on the seventh of September, 1934, carried streams of coarse sand and gravel to the foot of the bank. But one such stream met a sod two feet wide of *Polytrichum commune.* All of the gravel was stopped by the moss, nearly burying the plants. This sod makes a prominent knob on the face of the slide, with a vertical margin on the downward side. It has been catching gravel for many years. A stalk six and one-fourth inches long had only one and one-fourth inches above the sand, but was apparently in perfect health.

The capacity of mosses for holding mud is attested by raised peat bogs, where vast quantities of oozy peat are held by banks of Sphagnum. Such banks sometimes give way, and the black ooze pours out over the surrounding land. In northern Iowa bogs *Drepanocladus aduncus* forms are able to make small mounds filled with oozy mud.

The angle of slope that mosses can maintain ranges from vertical to horizontal. In studies of slopes it is essential to distinguish between those which are held by roots and peopled by mosses and those which are held by the mosses themselves. The latter only are considered here. Vertical walls of soil can be held only on small areas, or on soils that do not slump down when wet. Small (3 to 6 inches) vertical walls of fine soil were held at Cold Spring Harbor, L. I., by the mosses *Pohlia nutans, Dicranella heteromalla, Catharinea angustata, Ditrichum tortile vaginans,* and by the liverworts *Diplophyllum apiculatum* and *Lophozia excisa. Polytrichum commune* can hold an eight inch bank of gravel to a vertical position. Ofttimes soil washes away between small tussocks of moss, giving miniature bad-land topography on a centimeter scale, the moss serving as capstones to the pinnacles that remain.

A striking example of moss work is shown along Iowa River north of Homestead, west of Highway 149. All along the river for a quarter mile are mossy banks and knobs of *Anomodon attenuatus* and *Bartramia pomiformis,* several feet in extent. Occasionally a cushion of *Thuidium delicatulum* or *Hypnum patientiae* intervenes, or a bed of *Anomodon rostratus.* There are no gulleys in this long bed of moss. On gentler slopes *Brachythecium oxycladon* covers the ground. The top of this high bank, fifty feet above the river, is too steep and unstable for any plant life; it is nearly vertical. But stretching up toward this, ten feet beyond any other life, *Barbula unguiculata,* fruiting freely, has spread a thin carpet, and further washing is arrested. As a result of the absorbency of the abundant moss cover, this clayey river-bluff becomes water-logged, and great masses slump down, trees and all. But the resulting breaks are quickly covered again with moss.

Woodsy banks are held by *Anomodon attenuatus, Brachythecium oxycladon* and *Catharinea angustata* at Moore in Poweshiek County, by Anomodon and Bartramia near Kellogg in Jasper County, and at Eldora

in Hardin County, by Anomodon and Timmia at Monticello in Jones County. And great beds of *Mnium cuspidatum* hold firmly the shaded mossy knobs of Missouri loess north of Council Bluffs and in Waubonsie State Park at Hamburg.

The mechanism of sod formation by mosses (s. l.) is partly by a weft of protonema, partly by the branching multicellular monosiphonous rhizoids, and partly by the stems. *Pogonatum brevicaule* has a perennial protonema that covers many square feet of clayey road banks with a green felt in southeastern Pennsylvania. Such green films have been seen also on Long Island and in western North Carolina. In eastern North Carolina and in Florida *P. brachyphyllum* supplants the former species.

Liverworts hold soil chiefly by the felt of rhizoids. The collector slices off his specimens wtih a penknife, much as one might slice a piece of cheese. But this firm tissue is rarely one-fourth inch thick.

Most of the true mosses bind the ground with a mixture of stems and rhizoids, which may extend to a depth of six or eight inches. They may be divided taxonomically into erect stemmed Acrocarps and the creeping Pleurocarps, and each of these into inhabitants of aggrading areas and inhabitants of degrading areas. Aggrading mosses are able to grow upward as fast as the soil is washed in around them, up to a limit. Degrading mosses quickly succumb to a covering of earth. Many others, as those of rocks and tree-trunks, cannot endure any perceptible change of the substratum, and are of no interest here. The same is true of many pioneer species. Notable aggrading mosses are *Catharinea angustata, Polytrichum commune, Bryum caespiticium, B. bimum, Ceratodon purpureus, Amblystegium riparium, A. irriguum. Brachythecia* of the *salebrosum* group can creep out of a shallow covering of soil. Steep banks where degradational processes predominate are peopled by Anomodon and Bartramia, Thuidium and Hypnum. In general, the erect growth of Acrocarps is favorable on aggrading surfaces, and the creeping mats of Pleurocarps are suited to degrading surfaces. In both cases, soil movement is arrested, in the first by catching and holding the down-wash, in the second by preventing soil movement.

Unfortunately all moss mats on soils are easily broken by trampling by people and domestic animals. The mats peel off, carrying more or less of soil, and leaving naked spots or strips where erosion has no impediment. A single botanizing party of a dozen students can do irreparable damage. Consequently, in State Parks and preserves, visitors should keep, and be kept, strictly on prepared paths, wherever damage to ground cover is likely. And livestock must be kept out of hilly forest and erosion control projects.

In the 1898 Almanac of the Society of Agriculturists of France and in other horticultural manuals one may find directions for the *destruction* of mosses. Ferrous sulfate is the approved means. Directions for the *culture* of mosses are not easy to find. Nevertheless, on several estates on Long Island mosses of many species are being used in landscape work. An ecologist in charge of one large garden replied to a question as to the use of mosses, "Yes; easy to transplant and the very best thing we can get." It is customary there to transplant natural sods from out-of-the-way places, pack the pieces firmly into prepared soil and water thoroughly until the plants are established. No further care is necessary except to

sweep off fallen leaves. This is done at Great River by sweeping the miles of mossy paths weekly with a "besom"—a big, old-fashioned broom of slender twigs.

The indispensable requirement for growth of mosses is moisture, both of soil and air. It is necessary also to have partial shade, and a free circulation of air. There are also acidiphile species, neutrophile and basiphile species. Some can pioneer on new land, and others require more or less of humus.

Apparently there is no such thing as a prairie moss. The dense mat of grasses makes too much shade, and cuts off the air. And the quantity of dead material in winter completely covers the ground. Of course, if this litter is burned off, any moss must be destroyed. Certain it is, our efforts to find a prairie moss have so far failed. But between the tussocks of *Andropogon scoparius* in thin stands on Long Island *Polytrichum piliferum* and *P. commune* occur. Ceratodon takes hold on stabilized sand dunes. On wooded slopes in Iowa mosses abound wherever the cover of dead leaves is regularly blown away. And on exposed road-banks, especially on north-facing exposures, mosses often flourish.

As a pioneer on road-banks *Barbula unguiculata* has been found in Poweshiek, Iowa, Warren, Fremont and Dickinson counties. *Weisia viridula* and *Bryum sp.* accompany Barbula in Warren County. The ubiquitous *Ceratodon purpureus* is more permanent, if slower in coming. It makes miles of purple strip along the edge of the sod at the tops of road-banks in Linn County and elsewhere. On very wet ground *Mniobryum albicans* and *M. carneum* make soft sods. Raw acidic soils of Long Island accommodate Ceratodon, Pogonatum, Catharinea, Polytrichum. A little shelter of grass on Iowa road-banks enables *Brachythecium salebrosum*, *B. oxycladon* and *Catharinea angustata* to function as soil binders.

Whether these pioneer mosses can be encouraged in Iowa by artificial means awaits experiment. It is well known that any fragment of moss is able to put out protonema and new plants when placed under suitable conditions of moisture and light. It would seem likely that moss plantations could be started on many of our newly graded roadsides, and on erosion control projects by sprinkling on the soil and raking in a preparation of fresh moss material chopped by machinery into millimeter lengths. A very thin sowing of rye to furnish shade, and a favorable season, should bring the desired result. This is recommended for Ceratodon, Catharinea and Brachythecium. Spore sowings should be undertaken, with a nurse crop, for Ceratodon, Barbula, Weisia and Catharinea, all of which fruit abundantly.

SUMMARY

1. Mosses prevent erosion by catching the run-off water and suspended soil, and by matting the soil together and covering it so that it does not move.

2. This is accomplished by the mat of protonema, rhizoids and stems.

3. Valuable moss cover is delicate and must be protected.

4. Cultivation of mosses is practiced on Long Island, and suggestions are made for choice of species and procedures according to ecologic needs and conditions.

5. A table of significant species for Iowa is given.

APPENDIX: *Iowa mosses which are significant in control of soil erosion*

Short lived: 1 to a few years	Shade	Substrate	pH
Barbula unguiculata*	½	raw subsoil	calciphile
Bryum caespiticium*	¼	raw mineral soil	circumneutral
Mniobryum albicans*	½	wet raw subsoil	”
Weisia viridula*	0 to ½	raw or fertile soil	acid to basic
Long lived perennials			
Anomodon attenuatus	full	woodland soil and stones	neutr. to calciphile
” rostratus	”	” ” ” ”	acid to neutral
Aulacomnium hetero- stichum	”	woodland soil	circumneutral
Barbula fallax	½	raw subsoil	calciphile
Bartramia pomiformis	”	woodland soil	acid to neutral
Brachythecium oxy- cladon*	¾	fertile soil	calciphile
Brachythecium sale- brosum	”	” ”	circumneutral
Catharinea angustata	½ to full	” ”	circumneutral
Ceratodon purpureus	0 to ½	raw subsoil	acid to basic
Dicranella heteromalla	¾ to full	woodland soil	circumneutral
Hypnum patientiae	full	fertile soil	”
Mnium affine ciliare	”	woodland soil	mild humus
” cuspitdatum	¾ to full	fertile soil	acid to neutral
Plagiothecium deplana- tum	full	woodland soil	circumneutral
Plagiothecium roeseanum	”	” ”	”
Polytrichum commune	0 to ½	raw subsoil or fertile soil	acidiphile
Rhodobryum roseum	full	woodland soil	circumneutral
Thuidium delicatulum	”	” ”	”
” recognitum	”	” ”	”
Timmia cucullata	¾ to full	” ”	calciphile
Conocephalum conicum	full	raw subsoil or rock	”
Plagiochila aplenioides	”	woodland soil	neutral to acid
Porella platyphylla	”	” ” and rocks	circumneutral

*Ubiquitous in Iowa.

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CHALLENGE OF EROSION TO BOTANISTS

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From the University of Arizona

Almost overnight the term "erosion" has become a popular word. Used before in a restricted and often technical sense, it has now become as meaningless as the many political designations of socialism, communism, or Fascism. It is now used at random by business men, politicians, and uplifters. It has become in a sense a political shibboleth with miraculous significance, a safeguard of the future and a cure for the present depression. Possibly mankind can greatly profit by this publicity if such publicity makes available funds for a sane reconstruction and conservation policy. The erosion program is a great one and if it can have a rational approach may bring to bear practices which will protect our nation from a slow but sure self-destruction. Properly handled, erosion control is a great part of a sane conservation program.

Well-managed countries have no serious erosion problem. Europe, except for the Mediterranean region, has little to fear. Where land is given its proper use and where agricultural practices are well directed, this question need not be raised. Erosion is an indication of bad practice in forestry, range, pasture, or cultivated field. As weeds in cultivated crops are an indication of bad crop production practice, so erosion loss of cultivable land is a criterion of bad agronomic practice. On agricultural land the success or failure of cultural practices should be judged not alone by the crop produced, but by the absence of erosion damage to the soil and the maintenance of physical tilth and fertility. Good agronomic practice will see to all these factors.

Inter-relationships perhaps have not been sufficiently emphasized. Soil is as much a product of vegetation as vegetation is a product of the soil. The development of a soil, given proper basic material and a proper climate, is inconceivable without vegetation. Certainly the two great factors in developing a soil are climate and plant cover. It is almost inconceivable that our soils would have been what they are had they been subjected to cultivation from the start, or rather, had they been given clean cultivation. Damage to developed soils comes largely from removal of the crop and from long periods when they lie bare with no cover crop. If we were considering soil alone, this condition probably would not obtain, but the weed competition with cultivated crops may demand practices not best adapted to soil production or the maintenance of a developed soil.

On level or nearly level land destruction of the vegetation cover does not entail the same degree of surface loss as obtains on sloping land, the character of the soil material and the slope of the surface being the important factors. Superimposed on these two important factors are many incidental factors which often determine the amount of damage that will be done. To illustrate: the flat alluvial bottoms of the Southwest, covered with a coarse growth of sacaton *(Sporobolus wrightii)* may be flooded by

the runoff from adjacent watersheds. Over this flat land a great sheet of water, a veritable lake, moves down doing little or no damage, silting upon the flat and permeating the soil. A wagon track or cow trail may serve to start a defined current, which will finally cut a gully and in the end produce a channel, dry through much of the year but an eroding river during floods which will gradually cut out the whole area or lower the amount of water penetration on the areas on the side until they are no longer capable of producing a stand of sacaton. This type of erosion occurs in the giant rye grass (*Elymus condensatus*) flats of the Northwest. The damage comes from the establishment of the gully and when once well established on a large scale it is doubtful if it is practicable by any engineering works to restore the original condition. Similarly, a cut over timbered mountainside might not suffer destructive channeling if the undergrowth were not killed or burned and incipient channels developed by snaking down the logs.

Just as the occurrence of certain kinds of plants indicates bad agronomic practice on cultivated land so the appearance in pastures and range areas of certain plants or their increase in relative numbers indicates destructive grazing practice. At first the destruction may be only in the species constituting the plant cover and as a general rule the most valuable plants disappear first. Also as a rule less valuable or worthless plants, called weeds, take their place. That is, the first changes are qualitative and represent differences in composition rather than in density of plant cover. These changes often result in a loss in actual carrying capacity and unless stocking is reduced lead to serious over-grazing. This may be only the initial stage of destruction, and usually a short period of protection or of carefully controlled grazing will re-establishe the plant cover. Continued close grazing, heavy trampling, the formation of trails and channels which hasten runoff, all cut down the amount of water penetration into the soil, resulting in a decreased plant growth due to a lack of available moisture. The process is a rapid one, and soon the range will be irreparably damaged. These damaged ranges can be improved, but to develop a soil when it is once destroyed is not a matter of years or tens of years, but of centuries or thousands of years. Soils have been developed through long geological periods and once lost can not be regenerated in a few years. It is therefore imperative that no national government allow land to be destroyed in this way, be it public or private land. Similarly, the government should not allow fertile valleys to be dredged for gold for a present profit and left deserts of rock and gravel for thousands of years to come.

Our western ranges, especially on the public domain, must have immediate and constructive attention. The whole program is primarily a job for the botanist, be he ecologist, agronomist, or forester. Under all cases plants bind down the soil material and prevent its washing away. Erosion starts as a result of destruction of the plant cover. To re-establish this cover is a problem of agronomic or forest practice and generally must follow the course of secondary successions. Secondary successions as recognized by botanists are simply scar tissue in nature's attempt to heal a wound in earth's natural cover. The stages of re-establishment can easily be worked out, and in all cases recovery is most rapid when there is no further interference by partial or total destruction of the plant cover. To

remove continually the weeds from a deserted farm area on the high plains is to retard the re-establishment of the short grasses. But the presence of weeds may be a sign of improvement or a retrogression and only a trained ecologist can determine which process is taking place. It is all important if man is to direct the re-establishment of our depleted range land that he know the natural stages of revegetation in order that he may further the natural recovery rather than retard it.

Only a trained field man can determine the amount of damage done on the over-grazed ranges or to eroding soils. Except in rare cases plants are the only factors which can check erosion and aid in rebuilding the soil, and re-establish the carrying capacities of the range.

Wherever one looks, nature has pointed the way to recovery. Secondary plant successions mark the scar tissue necessary to heal the wound. And there are well-recognized steps in the recovery. On the high plains the storm troops are the annual weeds, the supporting troops the short-lived grasses and perennial weeds, and the final rehabilitation is established first by buffalo grass followed by blue grama. One must be able to interpret at once the significance of the weeds which enter. In the northern plains *Artemisia frigida* will dominate in about four to ten years and the grasses will be fairly well established in twenty years. On the southern plains *Gutierrezia sarothrae* will be established following weeds and short-lived grasses *(Schedonnardus texense)* in about eight to twelve years, and the short grasses not fully established inside of forty years. The succession is much the same on over-grazed land and on land plowed and allowed to revert. But over-grazing leads not first to the establishment of the early phases, but rather takes the vegetation back through the stages. Over-grazed lands in the North stand out conspicuously at a distance because of the silvery *Atremisia frigida,* and in the South green or yellow due to Gutierrezia. In the Central Plains Region it is usually a mixture of these two.

If I were to begin the job of correcting erosion damage, or better, of protecting the future from this damage in Arizona, there are six projects I would want undertaken at once.

First, a reconnaissance soil map made by, or under the supervision of, the best trained men of the Soil Survey working in cooperation with the local Land Grant college. This map should cover the forests, ranges, and Indian reserves, and include, at least in our State, land as yet not included in soil surveys. This map should show (a) depth and character of soil profile, (b) character of subsoil, and (c) character of undeveloped surface material.

Second, an over print indicating the amount of destruction of surface soil by erosion, fire, lumbering practice or any other known cause.

Third, an over print showing slope of soil surface.

Fourth, a vegetation map showing types of natural vegetation and an interpretation of the significance of each type in terms of agricultural, forest or range production.

Fifth, an over print showing the amount of destruction of native vegetation by grazing, logging, fire, cultivation, or any other cause.

Sixth, an interpretation of the five maps above based on the economic and social factors involved.

To make these vegetation maps men would have to be trained on the ground under the direction of a practical ecologist, one with range and

forest experience and one who had had some experience in mapping vegetation. Here the whole program might easily fail if placed in the hands of too theoretical a man, one who had written extensively but who had actually done no field mapping. It would likewise fail if, as often happens, it fell into the hands of too practical a man who had no respect for the opinions of others.

Botanists face this situation at a bad time. We have few men trained for the job. Those who are qualified are not likely to leave an established service to enter a public service which has shown greater disregard for length of service and qualification than ever before in our experience. Many qualified men are serving under those unqualified, which is the general rule in all political appointments. This condition should not continue under an administration which has so many wonderful programs in the making. Still, one can hardly blame those who wish to start a new line of work if they take new men, however poorly qualified, when we see how difficult it is to start new programs with the old machinery.

I still believe the Department of Agriculture and the Land Grant colleges know more about the problems of the land and the best future use of land and the problems of crop production, grazing and forestry than any other agencies in this country, that they have the best body of trained men, and that these men would be happy to launch out on any of these new programs if given a chance. Most of the programs have had to build up a personnel from this source, and the only new thing is the broader outlook and the transfer of men from department to department. No single incident has so delayed the development of a rational land use program in our country as the conflict between the Department of Agriculture and the Department of Interior. There are enough good men in both services to put over any program. The problems of the soil, agriculture, forestry, range, should all be in one department. The Land Office, what there is left of it, could probably be left or moved without consequence one way or another. But the disposal of the public domain by our present homestead method seems about at an end. It should be possible for our government to end this inter-departmental war for the good of the nation. Good men are equally divided in these two services and are equally capable, but politics has ruled the Interior Department and has been almost ruled out of the Department of Agriculture. Therefore Agriculture seems the only safe place for the great land use program.

The land use program is one of the greatest at present confronting our nation and the erosion problem only a symptom of failure in the past to have made the proper use of land. From a geological point of view and, in fact, from the standpoint of soil development, erosion is a necessity since very old soils are not the most productive. New material must be deposited or the old material rearranged, and erosion is the process by which this is brought about. Still the loss of the productive soils as a result of destruction of the vegetation which naturally protects them is to be avoided. This is the problem of the present erosion program.

In many parts of the world it is useless to attempt to slow down the natural processes. Where the total rainfall is insufficient to develop a dense enough plant cover to protect the soil the natural process of shifting surface material during heavy rains cannot be controlled. In each area the climax plant development under the climatic and soil conditions obtain-

ing will probably give the greatest protection. But over at least half of the earth's surface there is not sufficient vegetation to hold the earth in place.

A thorough understanding of the natural vegetation climax and of the secondary stages leading to its re-establishment, when it is once destroyed, is the best possible basis for a revegetation and erosion control program. Every location presents a new problem; every region requires special treatment and no one plan will apply when climate, soil and vegetation change.

Very briefly one may consider a number of cases presenting widely varying conditions:

1. *Sahara.* Sandy desert; here wind continuously moves the surface materials. There may be great flat areas of ripple-marked sand or great areas of moving dunes. Rain penetrates as it falls, but plants, although the annuals develop following rains and a few perennials maintain themselves, are never able to tie down the surface materials and develop a soil under the limited rainfall. Man could not improve this condition.

2. *French Somaliland.* A stony desert and heavy soil. Here rain does not readily enter the soil, and the rain water concentrates in lower areas to form water channels. These are often only slightly depressed below the surrounding surface. Along these water channels water penetrates sufficiently to produce coarse desert grasses, and these water channels are marked much of the year by straw-colored strips of dry grass. Here nature is immediately checking erosion by plant development. Man could not improve this condition.

3. *At Porto Libertad, Sonora, Mexico,* a heavy alkaline soil material is practically bare of vegetation except along drainage channels, and these are marked by a low, shrubby growth of Frankenia. Farther up, where more rain falls, Frankenia leaves the water channels and occupies the areas which lower down were bare, and the water courses are here marked by Atriplex. Man could not improve this condition.

4. *Cape Verde Islands.* The lee side of these islands is desert. Here are areas from which all vegetation has been removed. The natives go out over this area and pull up every living plant, place these plants in bags and sell them in the market as fodder. The rainfall is so light that it is doubtful if erosion could be prevented even if the plants were allowed to develop.

5. *The Growler Valley of Arizona.* Here there are no grazing animals, and there is no developed soil. The hills are covered with a sparse growth of cacti and desert shrubs. The ravines are marked by mesquite and ironwood and the flats by *Hilaria rigida,* a very coarse shrub-like grass, or are occasionally bare, with creosote bush surrounding the bare areas, indicating the absence of alkali even in the flats. Rainfall is very low, probably about five inches, and erosion not much retarded by the sparse plant cover. On a map showing erosion this area would show great damage, but there is no developed soil to be destroyed, and nothing can be done to prevent the washing

away of surface material. The condition is a natural one and even if erosion could be stopped no good purpose would be served.

6. *Omdurman.* Much of this portion of North Africa is grazed off by goats until one can form little idea of the natural plant cover. Crops are grown by flood-water irrigation and probably the whole area if protected from over-grazing would have an Acacia-desert grass cover. Great areas have become almost worthless as grazing land. Still the flat character of the land has not resulted in excessive erosion damage. Controlled grazing could here re-establish the grass cover and greatly increase the carrying capacity.

7. *Coachella Valley, California.* Water pours into this valley from the adjacent mountains during heavy rains, carrying with it great masses of earth and rock. Movement ceases almost as soon as the rains are over. The materials deposited in steep fans are sparsely covered with Atriplex, Gaertneria, Cycloloma, creosote bush, and many other desert plants. But this scattered plant cover plays no part in stopping the next flood, and the land of these fans may be moved at any time. There is no fixed soil. The floods are soon spread at the mouth of the canyons, and there is no gullying down the main valley, the water spreading out and sinking rapidly into the valley floor. Even small check dams would probably only increase the damage by breaking way during heavy rains. The earth is unstable and probably will remain so in spite of any revegetation or engineering program.

8. *The Mohave Reservation, Arizona.* This reservation is on the borderline between the northern (sagebrush) and southern (Covillea) deserts, the desert grasslands, the plain grasslands, and the Juniper-Pinon Woodland. In many places it is doubtful if a satisfactory plant cover could ever be established even with total protection from grazing. The better areas have been almost totally destroyed in places by concentration of sheep and goat herds. The sandy lands produce Ephedra and *Muhlenbergia pungens;* the better uplands *Hilaria jamesii* and *Bouteloua gracilis;* the alkali-free bottoms *Sporobolus wrightii;* the warmer and occasionally flooded flats *Hilaria mutica;* and the wet alkali flats *Sporobolus aeroides.* Here a number of imant and valuable grasses and shrubs such as *Atriplex canescens* are at home throughout the whole area. Each area is a natural habitat for one or more of the grasses mentioned above, and those persons directing the re-establishment should study them carefully throughout the natural range and know what their presence indicates in climate, soil, water supply, and damage by biological factors. Parts of the area can be greatly improved, but parts of it are not far below their natural condition. Erosion damage is not the only criterion of what can be done to restore such an area. The amount of damage to vegetation is the more important measure. How far has the vegetation been thrown back from its final stage? What position does it occupy on the scale leading from total destruction to complete re-establishment of the climax type? Only a field man of wide experience can determine this, and it is essential to an intelligent recovery program. The presence of a few remaining plants of *Hilaria jamesii*

should bring to mind the great areas of Nevada and Utah and the more restricted areas of Colorado, New Mexico and Arizona. Likewise *Eurotia lanata* and the plants mentioned above tell a wonderful story if their presence is properly interpreted.

9. *Lake Elton, beyond the Volga.* The so-called Artemisia steppes of Russia are not naturally Artemisia areas, for Artemisia is here a weed signifying over-grazing. It was probably a great grassland of *Festuca ovina* and other grasses, but so over-grazed for centuries by camels, horses, goats, sheep, and cattle that it now supports chiefly unpalatable and almost worthless Artemisia with a habit much like our *Artemisia frigida.* Here the carrying capacity could be restored, for the over-grazing on these great flat lands has not resulted in great soil loss. The surface is trampled and bare, but still the soil remains to be revegetated. The climate and soil could maintain a grassland, but it will probably require half a century to be fully re-established.

10. *The Sage Brush Deserts of Utah.* Somewhat similar to the creosote bush fans of the southern desert. Here for the most part the drainage from the watershed above seeps through the fan, but in times of flood tears a new channel through, destroying all vegetation and leaving a new scar to be tied down by the vegetation. Repeated burning and heavy grazing may result in complete destruction of the sage brush and vegetation cover and the fan being cut by a deep erosion channel. Under any system of reasonable management these areas will not require engineering work for their control.

11. *San Simon Valley of Arizona.* A desert grass cover washed during heavy rains by sheet water runoff on sloping land. The grasses retard the overflow, and the rooted tufts furnish sources of easy penetration. As a rule, water penetrates readily, and after a rain no water is left standing on the surface. The water spreads out in shallow, broad drainage courses where the added penetration suffices for a denser sod cover. Additional protection is thus given at the point most needed. Under natural conditions there is a continual tendency for the drainage channels to be tied down with mesquite, Bacharis, and coarse grasses, and the broad overflow channels to be held by a good grass cover. Over-grazing destroys the rate of penetration of water into the soil, and where vegetation is destroyed and the surface is puddled by trampling, increased runoff causes cutting along water channels and the ultimate gullying and destruction of surface soil of the whole area. Control of grazing is essential for either maintenance or recovery. Engineering work to spread the water may hasten natural recovery. Erosion destruction of surface soil can and should be stopped.

12. *The Tehachapi Valley of California.* Here a native bunch grass covered the slopes and hills and held the soil in place, producing a thick mat of vegetation with roots firmly anchored, so that a heavy rain could do little damage to the upper reaches, and only when water combined to flood the low places could damage be done. Now the perennial grass is gone, a heavy rain starts rivulets which cut at once into the soil, and the hills, which were once smooth and over which

sheets of water may have washed wtihout destruction, are now ribbed like a corrugated roof. Each rain increases the depth and length of the rivulet, until larger cuts are established. The soil, developed under a bunch-grass sod, is now being rapidly washed from place and carried down. The rate of revegetation here has not been determined insofar as I know, but it will, if we use the history of the great California grassland valley as a guide, never again be occupied by the original perennial grasses, but will each year grow an annual, short-lived weed grass growth which affords almost no protection at the beginning of the rainy season. The chances of protecting the area from almost total destruction passed when the last of the perennials were killed out. Revegetation is always possible, but here seems to be exceptionally slow. Here protection of the soil is a matter of retaining the grasses, and these can only be retained by preventing excessive grazing.

13. *Sacaton and Elymus Flats.* Sacaton in the southern desert and Elymus in the northern desert are two great grasses which covered the rich bottom soil piled in by erosion from the adjacent hills. Neither grows on alkali and therefore they are confined to well-drained bottom lands. Floods from the hills, even with a considerable amount of silt, could spread out over these flats, and although the slope might be considerable, the coarse bunches of grass hold against soil cutting. Many of these great bottoms are now changed to a wide sandy wash with banks occupied by desert growths of creosote bush in the south, and sagebrush in the north. The washes are cutting down the banks and widening each year.

There are probably two chief factors at work here. The overgrazing of the higher lands adds to the bulk of the flood water. This alone would probably have added to the density of growth of the sacaton or Elymus, but fire destroys the tops of these grasses when dry and possibly improves the value to grazing animals in making the young growth available. Fire and over-grazing have destroyed much of the grass in these great bottoms. The water has cut channels and the flooding or natural water spreading stopped. These flats have disappeared. So great is the volume of water poured through some of these big washes that to spread it out as formerly seems an almost hopeless task. Most of the engineering is done to force retention in the present channel. This seems nearly an impossible piece of work. Control of grazing and fire would easily have avoided the present condition. One can see no easy or practical method now of securing a return to the original condition.

14. *Tobosa grass flats.* On relatively flat land in Arizona and New Mexico, where water flows over and stands for a time on the surface and then slowly penetrates, Tobosa *(Hilaria mutica)* forms a dense, wiry mass. It is not easily killed out, but can be completely destroyed by heavy grazing. Naturally no erosion will take place here and if protected, the grass cover will in time be established. It is probable that artificial means could be employed to greatly increase the rate of recovery.

15. *Tussock grass flats.* These are similar to the above but generally require more water and an alkali land. This *(Sporobolus aeroides)* is one of the best grazing grasses of the desert regions and with only moderate protection can be retained in a productive state. These areas will not successfully produce any other grass.

16. *Short grass and desert grass plateaus of New Mexico.* A sparse cover of *Bouteloua gracilis* and *Bulbilis dactyloides* in eastern New Mexico maintained itself until badly over-grazed. The same is true of black grama *(Bouteloua eriopoda)* grass areas on the desert plains. Now these grasses have been largely replaced by Gutierrezia, and the range cannot be returned to its original carrying capacity for many years, probably twenty to forty years at least. Similar areas in Arizona are almost useless as range land when compared with the original condition. Heavy grazing during dry years has resulted in this destruction. Careful range management would have richly rewarded the rancher who practiced it. Protection and careful management could improve this range, but the recovery will be exceedingly slow.

17. *The John Day country of Oregon.* A short growing season and a grass cover of high palatability, characterizing grasslands with forest boundaries such as we find in the John Day country or, for that matter, at the V. T. meadow on the Kaibab in Arizona, offer a condition in which the best species can easily be killed out and the less palatable and desirable species like Antennaria take their place. Often total destruction leads to loss of surface soil, but in all cases the production of feed is soon greatly reduced from a carrying capacity of 300 to 600 sheep per section to a small fraction of that number. Careful management of this range and control of grazing will maintain a high carrying capacity and protect the soil from erosion damage.

18. *Sand lands of eastern Colorado.* Here heavy grazing and trampling, especially about watering places, may start blowouts by entirely destroying the vegetation. Heavy grazing may kill out the taller grasses and reduce this Andropogon grassland to a short grass cover of Bouteloua. Still, it is hard here to destroy the vegetation entirely, and the reduction of the carrying capacity and increase of unpalatable *Artemisia filifolia* is the chief result. Here control of excessive grazing would lead to good results.

19. *Badlands of the Dakotas.* These badlands were not caused by overgrazing, but are the result of natural erosion. The surface soil is destroyed and again established at a lower level. In places five developed levels can be found, the short grass fully established and the soil profile developed at each level. It is doubtful if any amount of engineering or range control could stop this condition. Although it looks much like induced erosion damage in the South, it is a more nearly natural phenomenon.

20. *The high plains.* This year parts of Kansas were picked clean of vegetation as a combined result of drought and heavy grazing. Occasionally the gentle slopes have shown erosion channels, but these chan-

nels have been immediately filled with Russian thistle and will be promptly tied down by this plant more effectively than would be possible by a great amount of engineering work. There is little danger of water erosion on the high plain. Wind abrasion of clean cultivated fields is common, but even here the remaining soil material proves almost as good for crop production as the natural surface soil.

21. *Tallgrass lands of the Transvaal.* A destruction of this grass cover results in a secondary succession leading back to a re-establishment through much the same stages as occur in our high plains, but this return seems to be more rapid. Burning, followed by close overgrazing, will rapidly destroy the grass cover. Burning is therefore being discouraged by the best practices. Good management will insure protection of soils here by a dense grass cover.

22. *Yellow pine land* is usually protected by a scattered growth of grama grass, Poa, Muhlenbergia, and many small herbaceous and woody pants. The cutting of yellow pine does not result in a great change of the ground cover, but as a rule there is no great danger of erosion destruction over the yellow pine areas if the grasses are not destroyed by over-grazing.

23. *Spruce-fir lands.* Here the openings are covered with a grass and herbaceous plants which are easily destroyed, but the destruction of the forest results usually in a rapid re-establishment of fireweed, aspen, grasses, and finally the original forest. So rapid is this recovery in the early stages that unless fires are continually repeated or over-grazing permitted, the vegetation will prevent erosion loss.

24. *Oak forests of Maryland.* A field left fallow will usually be covered with a fairly dense weed growth the first year, and little damage will result from erosion if the vegetation is protected. Soon Andropogon replaces the weeds, field pine the Andropogon and the pine is replaced by oak in less than a century.

25. *Cut-over pine lands of Michigan.* Here land cut over and burned repeatedly has been so depleted that only with difficulty can pine be re-established. Still, this sand land produced most valuable forests under natural conditions, and this productivity could have been maintained by proper methods of forestry. Here the soil has been destroyed, not by erosion, but by continual fires.

26. *Tropical rain forest.* Destruction of this forest requires a combination of cutting and burning and usually cannot be accomplished inside of one or two years. Even if reduced to clean cultivation it rapidly returns through well marked stages to a forest type. There is little danger of soil loss.

27. *Temperate rain forest.* A great and varied forest occupying for the most part steep mountain sides. Here crops are grown for a few years. When abandoned, it is rapidly covered by bracken and brush and vines and returns to a forest type. So rapid is this re-establishment of plant cover that erosion damage is very slight.

28. *Mountain grasslands of Central Africa.* Here soil conditions are much the same as in Temperate rain forest, and these grasslands are probably the result of destruction of that forest followed by frequent fires. Here the soil may erode away as in some of our southern states. Usualy this erosion is started by paths or over-grazing or some unnatural cause. Protection of the grass cover would entirely prevent destruction of the soil under this type.

This brief resumé of conditions in a number of widely separated regions serves only to show how different must be the method of attack if we are to prevent damage to already well developed soils. It should also indicate caution in undertaking improvement where there is little likelihood of success and where no useful purpose would be served by attempting to control erosion.

With no desire to minimize the need of engineering work in connection with flood control and the control of erosion damage, it is to the agronomist, the forester, and the range ecologist that we must look for the major part of the work in the solution of this problem. In other words, it is largely a matter for the botanist who knows and understands the functions of the various plants, in the formation and holding in place of the soils in question. Secondary succession, an almost academic study a few years ago, must become of major practical importance if we are to return our non-cultivated lands to a stable condition. To maintain our cultivated fields, our agronomists must look to the problem of protecting surface soil from loss by sheet or gully erosion. The problem confronts us today becase we have failed to develop the proper practices in the years gone by. The seriousness of the question of soil erosion is commensurate with the degree of our neglect.

NATURAL REVEGETATION ON ERODED SOILS IN SOUTHEASTERN OHIO

J. A. LARSEN

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It is well known that on rolling lands where the plant cover is seriously disturbed by farming practices erosion frequently becomes very rapid and destructive. The purpose in this paper is to set forth certain changes which are taking place in the vegetative cover on severely eroded and abandoned farm land in southeastern Ohio. This land had been used for livestock farming. Due to its topography, certain tracts had been kept in permanent pasture and the more level land in continuous cultivated crops. As a result, most of the upper A horizon has been lost by erosion. The pasture areas had deteriorated through overgrazing, scant fertilization and improper rotation until the more palatable grasses have disappeared, giving place largely to weeds and grasses of little forage value. In this study a 30-acre unit on one of these farms was mapped, defining the forest areas with the shrub borders, the various types of grasss and the land defaced by gullies. Transects were made through types of forest and shrub associations and quadrats were used for a more detailed study of the herbaceous communities. Near each of the quadrats soil profiles were obtained in order to show the relation of vegetative cover to the extent of erosion.

LOCATION AND DESCRIPTION

The particular farm studied passed out of cultivation twenty-five years ago. It lies within Salt Creek Watershed five miles northwest from Zanesville in Muskingum County in the dissected plateau between Licking River and Timber Run, Section 19, T. 1 N; R. 8 W. (Falls township).

This is in the more subdued western part of the Allegheny Plateau where the land has been maturely dissected until it consists of variously shaped blocks and remnants of table land. Here and there are narrow ridges and somewhat steep-sided valleys from 200 to 300 feet deep and rather limited bottoms. The soil was originally a reddish silt loam from 6 to 8 feet deep in the A horizon and from 15 to 20 feet or more of rather heavy silt loam in the B horizon.

RESULTS

By natural drainage units the land embraced in this study may be placed in three distinct divisions, slope No. 1 toward the north, used heavily for cultivated crops; slope No. 2, toward the east and old meadow; and slope No. 3, a southwest aspect the upper and lower portions of which were once in cultivated crops with pasture in between on the steepest part (Pl. I, fig. 2). The gradients are generally slight on the upper part of the slopes, rather steep with 25 to 30 per cent gradients at the middle

FARM IN MUSKINGUM COUNTY, OHIO.

Legend

FOREST | Sumac | Dewberry | Erosion | B Bluegrass | A Andropogon Beard Grass
High Shrub | Tall Blackberry | W Weeds | Contour | R Red top | D Poverty -

Fig. 1. Map of the abandoned farm where cultivation ceased 25 years ago.
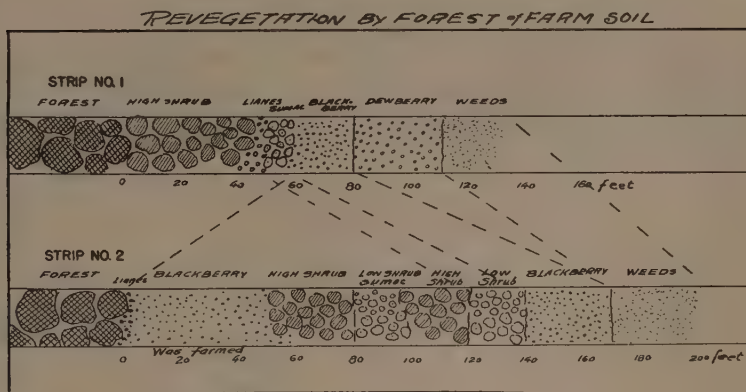
Fig. 2. The aggrading series in the shrub border.

sections and decreasing again to 10 or 12 per cent and less near the toe or bottom.

At present the native vegetative cover falls into several distinct communities and associations (see map fig. 1 and table 1). It should be noted that the numbers 1 to 7 in table 1 represent revegetative or aggrading seres, processes which will eventually arrest erosion when grazing is excluded, and that the numbers 8 to 11 are communities of native grasses which present degrading seres that will yield place to the invading shrubs and forest trees. The series of the native grasses reflect deteriorating soil conditions or stages of soil erosion.

TABLE 1. *Present vegetative communities on area mapped*

Community	Acres	Percentage
1. Forest	8.19	26.8
2. Tall shrub (sassafras, etc.)	.25	.8
3. Low shrub (sumac, etc.)	1.05	3.4
4. High blackberry	3.37	11.0
5. Dewberry	1.05	3.4
6. Weeds	3.48	11.3
7. Gully erosion	3.39	11.2
8. Poverty and beard grass	6.29	20.5
9. Red-top	3.07	10.0
10. Blue-grass	.50	1.6
Total	30.64	100.0

Note: The laying out of the tract itself in respect to hill tops, slopes and valleys would affect the proportion of each unit or community to a very large extent; for example, if the plot had been extended eastward over the higher ground and top of the hill a much larger proportion of the blue-grass would have been included. This relative proportion of blue-grass is not significant and not represntative of the true relation that this community bears to the other grass communities.

THE FOREST AND FOREST BORDERS

The wooded areas which constitute the aggrading seres are now in two locations; the bottoms, where at the time of actual farming operations a few scattered trees of white oak, white and red elm, black walnut, shagbark hickory, black gum, willow, poplar, etc., remained; and the hill-top above slope No. III.

ROOT SPREAD OF DEWBERRY.
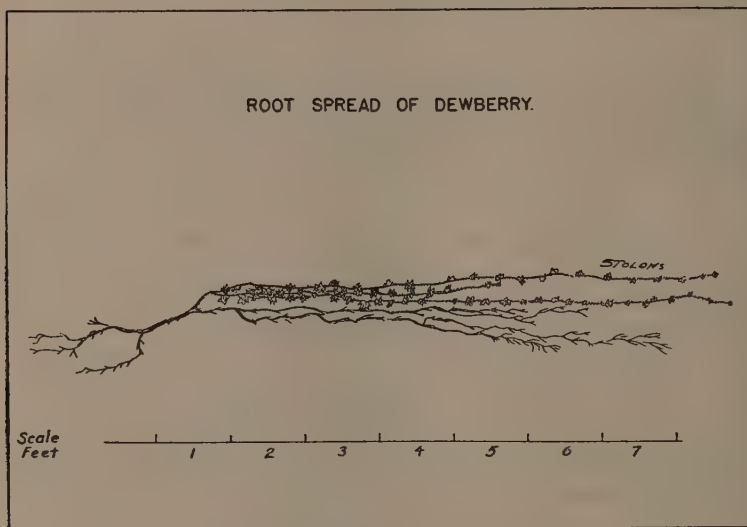
Fig. 3. Roots and stolons of dewberry.

Nut trees do not depend upon the advance of the low or high shrub border to become established, but are seeded directly by squirrels and thus pioneer to considerable distances from the forest or woodlot. Hickories usually spread by this means downward over southerly slopes and sometimes upward the same from trees standing below. Black walnut is extending upward along the more moist valleys or bottoms, the seed being carried from trees which remain on the lower parts of the slope or in the draws.

Other forest trees than those mentioned above except oaks and elms, do not generally invade open and exposed areas on poor soil unassisted by the shrubs. The high shrub border which stands between the trees and the low shrub sere is composed of many dwarf treees, and tall shrubs, sassafras being one of the most common with varying proportions of hawthorn, wild plum, etc. The low shrub border, which advances ahead of the tall shrubs, runs heavily to sumac (*Rhus glabra* L.) hazelwood, (*Corylus americana* Walt.), red root (*Ceanothus americana* L.), high blackberry (*Rubus allegheniensis* Port.), and roses, etc.

In most cases when the shrubs originate in the open away from the timber the first plants are cinquefoil (*Potentilla procumbeus* Sibth.) and dewberry (*Rubus procumbens* Muhl.) These two are almost invariably found together and are very active and efficient in covering exposed soil

of low fertility. They drive out the weeds and prepare the ground for the low shrubs, which in turn pave the way for the tall shrubs and eventually the trees (fig. 2).

The natural sequence of the succession, therefore, whether the revegetation takes place from the forest as a border or independently out

ROOT SPREAD OF BLACKBERRY.

Scale
feet   1   2   3   4   5   6   7   8
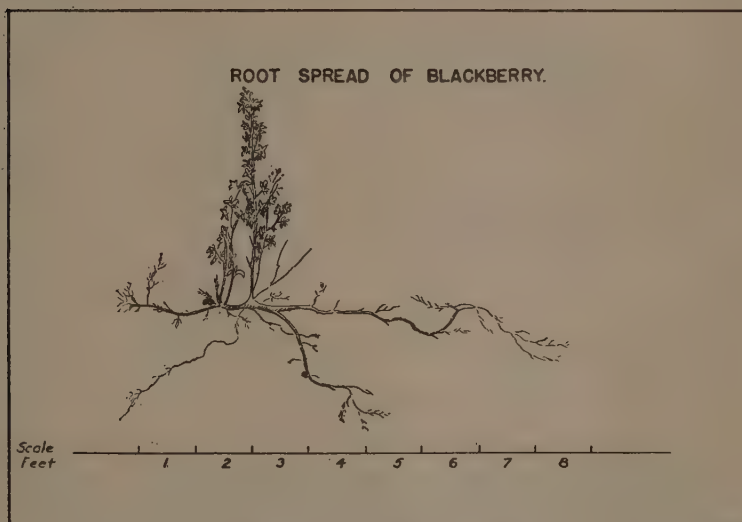
Fig. 4. Root habit of blackberry.

in the open, is: (1) dewberry and cinquefoil; (2) rose, hazlewood and high blackberry, etc.; (3) tall shrubs and dwarf trees, and (4) the forest. Black locust and Ailanthus frequently function in the tall shrub border. The former much more often than the latter, especially of dry sites.

Tillage has in many instances interrupted the natural and regular orientation of the successional belts or seres, rendering it difficult to recognize the true relations. Nevertheless, the trend is there and the progression continues.

It is fortunate that practically all of the low shrubs and sassafras are propagated by birds and spread by root runners or root shoots. This makes them doubly efficient in driving out the blackberry and preparing the site for the trees. Sumac, especially, takes root readily everywhere on dry land and multiplies rapidly (figs. 3, 4 and 5). Annual roots of the blackberry have been measured up to six and seven feet in length. From observations on other areas within the county it is concluded that this species reclaims cleared forest land with great rapidity, following the early succulent weeds in short order and occupying the land for a time (fig. 4). Oaks and hickories are sometimes seeded by squirrels among the berry patches.

Dewberry (*Rubus procumbens* Muhl.) in most cases appears to be a forerunner of the high blackberry. It also is seeded by birds in new locations and spreads both by trailing stem and by root sprouts. The trail-
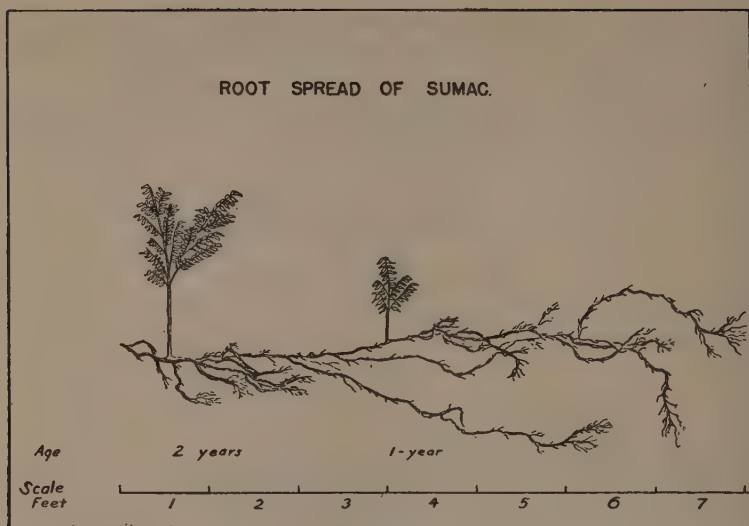
Fig. 5. Root extension of sumac.

ing stems may grow nine feet in a season, take root, and become centers for spread the following season. Cinquefoil, with which dewberry is often associated, spreads in the same manner, but not so rapidly. Both of these species advance in pure and dense formation and will grow with less moisture and on poorer soil than other species. For these reasons they become very important agents in reclaiming wornout farm lands.

Weeds of the fields are less desirable and less effective plants for erosion control than grasses. The more common weeds on such areas are the goldenrods, asters, long-bracted plantain, ragweeds, sheep sorrel, Canada blue-grass, wild carrot, yarrow, poverty grass and triple awn grass (table 2). Though the different species differ in point of time of most rapid growth, root and top development, grazing value and xerophytic rating, some of them serve useful purpose in revegetating the smoother slopes, the heads of gullies, and areas around dams.

Many species of weeds are able to tolerate very poor growth conditions, often persisting where erosion has progressed deeply into the B horizon. However, where they grow on the poorest soils, they are relatively ineffective in preventing sheet or gully erosion. In addition, many of these are annuals and therefore are undeveloped when much of the heavy rainfall occurs.

THE NATIVE GRASSES

Slope I, formerly used for clean cropping, has now been largely reclaimed by the various native grasses. Relative tolerance of these grasses to soil and surface conditions as shown on this slope and elsewhere throughout the Salt Creek watershed has resulted in a degrading series

TABLE 2. Data from one quadrat in each community

Species	Blue-grass community		Meadow		Bluestem poverty grass		poverty grass		Weeds— poverty grass	
	No. plants	Area dm³	No. plants	Area dm³	No. plants	Area dm³	No. plants	Area dm³	No. plants	Area dm³
Blue-grass *Poa pratensis* L.	102	49.82								
Yarrow *Achillea millefolium* L.	30	9.57	8	0.72			19	1.67	5	0.70
Sheep sorrel *Rumex acetosa* L.	14	3.36	54	6.01	3	0.94	5	0.48		
Panic grass *Panicum capillare* L.	8	3.41	20	6.37			8	0.76	7	1.87
Beard grass *Andropogon virginicus* L.			23	13.88	17	15.12	17	7.86		
Aster sp.			19	5.28						
Wild carrot *Daucus carota* L.			15	4.51					7	1.34
Golden rod *Solidago riddellii* Frank			9	1.51	2	1.00			5	0.70
Pennyroyal *Hedeoma pulegioides* L.			9	1.06	7	1.94	15	1.09	5	0.63
Foxtail *Setaria glauca* L. Beauv.			19	2.11						
Poverty grass *Danthonia spicata* L. Beauv.					54	4.81	70	12.37	28	4.01
Ragweed *Ambrosia artimisifolia* L.					1	0.28				
Spurge *Euphorbia* sp.							12	0.80		
Plantain *Plantain lanceolata*									45	7.67
Miscellaneous weeds			11	2.63	2	0.43	17	2.16	20	4.03
Miscellaneous grasses*									5	0.64
Total No. plants	154		195		80		163		127	
Percentage area covered		66.16		45.41		24.52		27.19		21.59

* Orchard grass.   Canadian blue-grass.
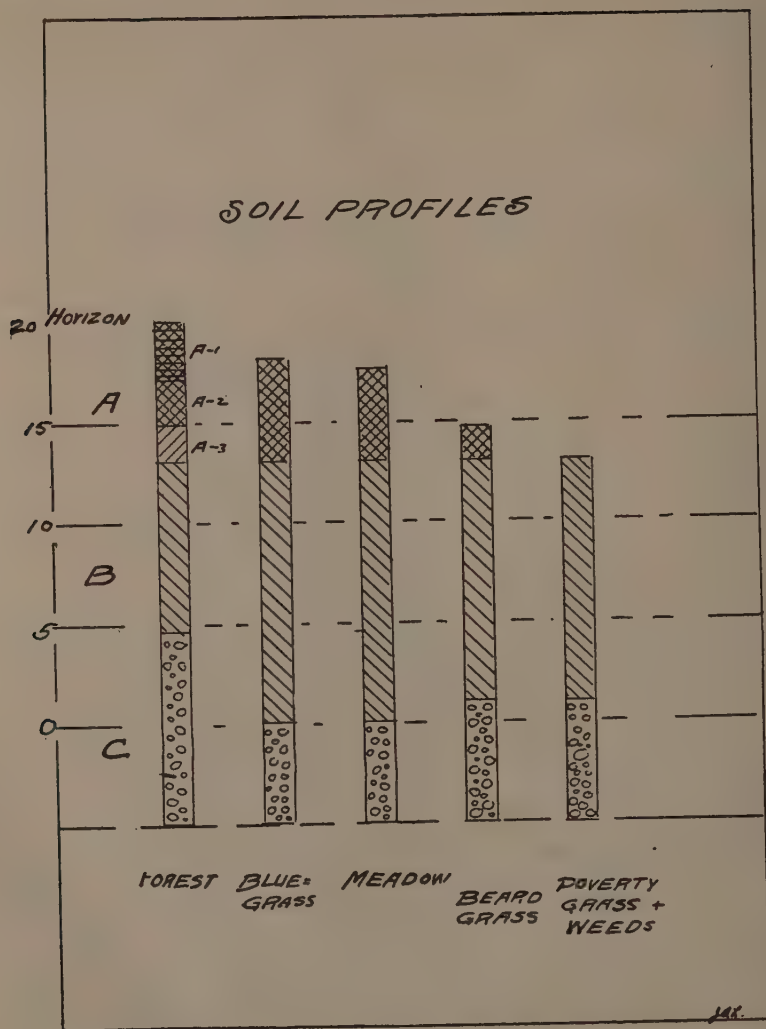
Fig. 6. The soil profiles under the degrading series of native grasses and weeds.

from Kentucky blue-grass to poverty grass and weeds. Where the soil of horizon A is still present in considerable quantity, blue-grass (*Poa pratensis* L.) usually invades. On the upper location on Slope I, Kentucky blue-grass occurs, in some spots mixed with small groups of Canada blue-grass. Directly below this is a zone of red-top (*Agrostis alba* L.). Its posi-

tion is near the shoulder and upper part of the hillside. Below the red-top belt are dense stands of beardgrass, also called bluestem (*Andropogon virginicus* L.), with much triple-awned grass (*Aristida oligantha* Mich.) on newlyburned patches. On the lower part of the beardgrass strip and encroaching upon the lowest belt, the weed association, was a belt of poverty grass (*Danthonia spicata* L. Beauv.).

This vertical stratification of the native grasses is evidently an index of similar contour strips of soil conditions. Stewart (2) has studied the chemical and physical conditions of the soil upon which these different grass associations thrive and has found a definite relation of higher acidity, less water absorption and less available nitrogen and phosphorus in the soil covered by the grasses of lower palatability. The soil on which blue-grass is growing is the closest to the neutral in this series, and the soil on which poverty grass is growing the most acid of the series.

During the summer the writer made an attempt to map certain quadrats of representative grass compositions. Two of the five quadrats were laid out on the Northwestern Appalachian Soil Erosion Experiment Station, the next two on slope I, and the last on slope III on the farm where the studies in spread of shrubs were made. The results are given in the form of graphs and tables in the following pages. (It must not be supposed that these plots, chosen with the idea of picturing the conditions, represent actual averages.) The series show a fairly regular decreasing density of ground cover from the blue-grass to the poverty grass and weed stages. By a correlated sampling of the soil presented in profile there appears also a degrading sequence from the hardwood and blue-grass soils to that of the poverty grass and weed soils.

Where Kentucky blue-grass is growing, the absorption is enhanced by a porous structure of the soil, particularly of the A horizon. The arrested surface flow here is also instrumental in favoring absorption of water. In the case of weed areas and poverty grass, the thin cover allows the rain water to rush over the surface, and less porous soil prevents rapid absorption. The difference in the absorption of rain water by these soils was brought out forcibly by measurements of water penetrations following the heavy rains of August 2. After 2.38 inches of precipitation during the early hours of the morning, it was observed that the penetration of soil in the hardwood timber was eight inches, in blue-grass soil seven inches, clean culture of corn five inches near the plant itself and three inches elsewhere, on bare, badly eroded soil from one and one-half to two inches.

In looking over the quadrats and tables remarkable differences are found in the ground area actually covered by these plant associations. In case of the blue-grass plot the percentage of the total area occupied by the vegetation is 66.16; in the poverty grass plots from 24 to 27 per cent; and in the weed plot 21 per cent. It is clear that when the soil has been depleted by heavy grazing, in the process of which the more palatable grasses are gradually eliminated and the poorer ones left to reproduce, erosion readily sets in, the result being that gullies are developed. This is the condition found on slope I.

The differences brought out by the density charts bear directly on the grazing quality or the carrying capacity of the land. Clements (3)

has listed forage values for these grasses showing that in the matter of ash content, ether extracts and crude protein composition *Poa pratensis* ranks higher than Aristida, Andropogon and Danthonia.

It would be misleading to present actual figures on the carrying capacities of the range as represented by these plots. However, it is a safe assumption that the blue-grass pasture, by virtue of its greater coverage, more rapid growth, and greater nutritive qualities, would support from four to five times as many head of stock as the beard grass-poverty grass pasture. From the standoint of evaluating the range in carrying capacity we should bear in mind the matters of density, composition, rate of growth and nutritive value of the grasses. The natural result of poor pasture management is, of course, a change in the composition of the grasses toward the least desirable and to weeds, and the invitation of sheet and gully erosion.
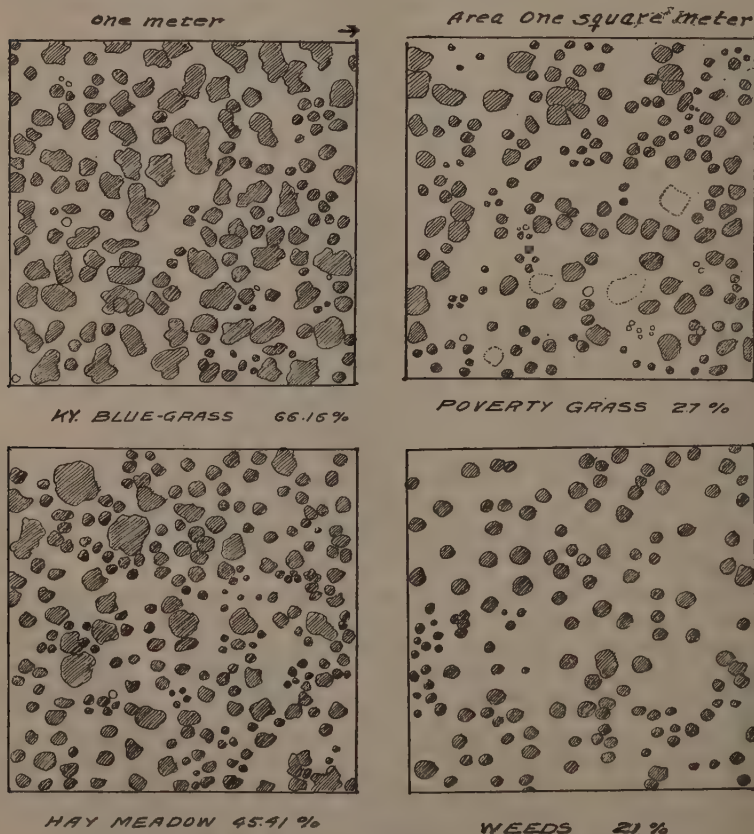
Fig. 7. Density charts from quadrats in native grasses and needs.

In the maintenance and rehabilitation of the pasture, the Agricultural Extension Service in Ohio has shown that complete fertilizers and phosphates in particular are very effective, and that an application of from 150 to 375 pounds of complete fertilizer per acre increases the production of beef and milk and the density of the vegetative cover materially.

The prevalence and great abundance of the Andropogon or bluestem areas throughout the Salt Creek watershed may be explained by the low palatability and poor succulence of this plant. Palatability is of great importance in determining survival of a grass. A plant of relatively high yield and nutritive content may remain untouched in a community of more palatable species, while it may be completely utilized when it forms a pure community or stand or occurs in the absence of more succulent forage. Bluestem is seldom eaten when other grasses occur in the mixture. As a consequence foxtail, blue-grass, legumes and succulent weeds are removed from the range. An increase in the proportion of beard grass and poverty grasses is the inevitable result. It is not unusual to see entire fields completely covered with a dense, pure stand of this grass. The poverty grass and triple awn grass represent the poorest grasses of general occurrence. They are very low in nutritive value and palatability, occurring mostly on the exposed B horizon where the land is not even suitable for many of the common weeds.

Lichens grow on some of the driest and most sterile locations, often being the first pioneer plants in such situations. Where weeds and poverty grass cannot survive, lichens enter, provided erosion is not active. These plant combinations of algae and fungi exist because of tolerance to drought, efficiency in water absorption, and through solution of minerals in rocks by carbonic acid formation. During the severest droughts they pass into a resting stage. Suitable habitats for these plants occur along the edges of breaks of gullies and at the lower fringe of the weed and poverty grass communities.

GULLIES

The most critical parts of the area from the standpoint of erosion are the deep gullies which are spreading at the present time because of the sparse plant cover, the lost A horizon and continuance of sheep grazing. Here the rate of erosion has progressed faster than plant invasion. During the last year the sheep have been excluded and some planting of black locust and Norway pine attempted in the worst gully. Judging from the success with black locust planting in similar situations on other farms in the vicinity it appears that erosion can in this manner be permanently arrested. The healing and revegetation is naturally hastened by construction of check dams.

SUMMARY

Many farms in southeastern Ohio where intensive cultivation and pasturing have been in progress for the last 100 years or more show in most cases severely depleted surface soil and a distressing degree of gullying.

Studies which were made on one farm which has not been cultivated for about twenty-five years show unmistakable trends and tendencies in the rate and manner of revegetation and reclamation by the invading seres of the native vegetation.

Where cultivation and grazing have been discontinued the natural vegetation reclaims the depleted areas at a very encouraging rate, tending to rebuild the eroding soil. In the process of rebuilding or aggrading, the first species to cover the surface are the cinquefoil and dewberry. These are followed by high blackberry and later by a low shrub border of sumac, hazel, rose and other species. The low shrub border gives way to the tall shrubs or dwarf trees and eventually to the forest trees. The spread of the forest is up the hill from the bottom or downward from the hill top according to the species in regular successional aggrading seres. The forest trees also seed in where scattered nuclei of shrubs have taken hold in the open fields. The low as well as high shrub border are very aggressive in that they spread by root extension and suckers advancing ahead of the forest into the open and abandoned areas now occupied by weeds or poverty grasses.

Old fields and like areas show stratification of the reclaiming grass communities in the degrading series from Kentucky blue-grass to red-top, bluestem, poverty grasses and weeds. The first of these is found where the deep and porous loamy soil has remained and the last seres on terrain devoid of A horizon soil.

By virtue of the denser vegetative cover of about 60 per cent and the porous nature of the soil underneath, the blue-grass areas absorb considerably more of the rain water than the soil covered wiht poverty grass or weeds. Naturally, where less of the rain water is absorbed by the soil more of it runs over the surface and becomes instrumental in causing erosion. For these reasons we find erosion gullies developing to a maximum degree on areas covered by weeds and poverty grass.

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PLATE I

Fig. 1. The topography is in the nature of a dissected plateau with most of the cultivation on the table lands and bottoms; timber and pasture on the slopes.

Fig. 2. Abandoned farm revegetating from the forest on the hilltop and from the bottom stream.

Fig. 3. The eroded hillside where years of clean cropping has reduced the A horizon, increased the native grasses and weeds which do not hold the soil.

PLATE I
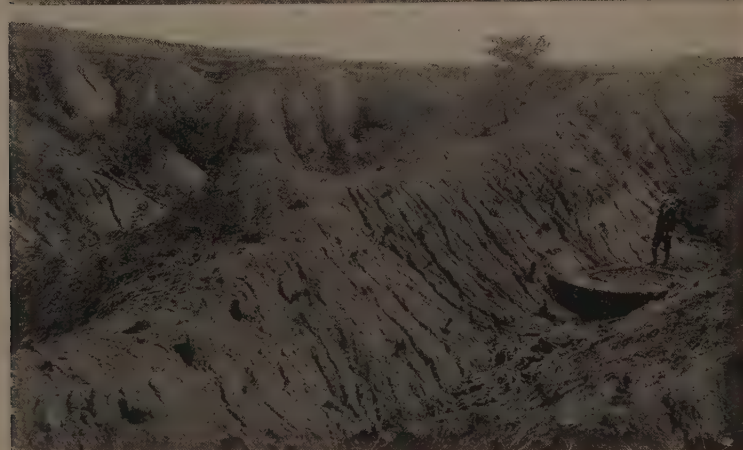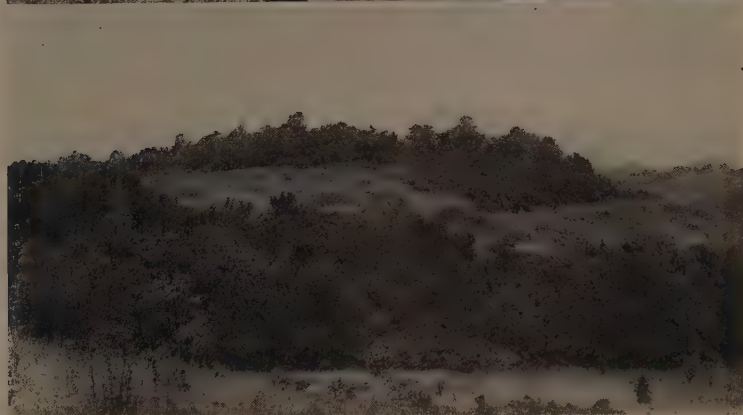
THE RELATION OF THE STAGES OF PLANT SUCCESSION TO SOIL EROSION

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From a study of geological evidence the conclusion may be drawn that the surface of the earth was at one time devoid of plant cover and that it gradually became covered with vegetation by the development of successive stages of plant growth from simple to more complex types. Observations of the development of vegetation on bare surfaces in short periods of time, give added weight to this conclusion.

To facilitate such studies the process known as plant succession has been arbitrarily divided into stages based on type of plant cover present in each stage. Since, as has been established in general, the rate of soil erosion decreases with the increase in total plant cover, it may be assumed for the purpose of our study that each succeeding stage of plant succession, which results in an increase in plant cover, has a greater erosion-prevention capacity than the preceding one. The purpose of this paper is to report the results of a series of observations made upon a number of sites representing stages in plant succession, to determine the extent of changes induced by the plants which may be effective in preventing erosion.

MATERIALS AND METHODS

The early stages of plant succession on rock surfaces are separated in time by periods of hundreds to many thousands of years and the later stages by tens to hundreds of years, depending on the structure of the substratum, the climate and the plants involved. In our study sites were selected which are separated in space rather than in time, but which represent stages in regular succession. Many such sites were available on the sandstone ledges and on sandy soil resulting from their disintegration in The Ledges State Park which is situated about four miles south of Boone, Iowa; from these were selected sites typical of each stage of the succession. At least some of the sites representing each stage were on slopes as steep as 45 per cent. The diagram in figure 1 is a graphic presentation of the stages of the xerosere represented by the sites selected. The tree stages which are shown as the climax in the area studied, are considered by some to be post-climax stages induced by the more favorable habitat on slopes bordering streams.

Habitat changes, induced by the development of vegetation, which may be considered most important in the prevention of erosion, were investigated as follows: (1) percentage of plant cover; (2) depth of soil; (3) water-holding capacity of the soil; (4) wilting coefficient of the soil (the percentage of water remaining in the soil at permanent wilting of the plants); (5) total organic matter in the soil.

Percentage of plant cover was computed by the total-square method from quadrat charts plotted on engineer's graph paper. The area of the

quadrats studied was one square decimeter for the lichen stages, and one square meter for the later stages.

Water-holding capacity of undisturbed soil was determined by forcing into the soil a metal cylinder and removing a core of soil, one square decimeter in area and of one decimeter in depth and measuring the quantity of water in cubic centimeters which was held by this core against gravity. For comparison with these results, the laboratory method was employed in obtaining the water-holding capacity of coarsely screened composite samples of soil taken from each site.

The hygroscopic coefficient was used as a basis for the computation of the percentage of water remaining in the soil of each site at the sage of permanent wilting of the plants. The formula used:

$$\text{Wilting coefficient} = \frac{\text{Hygroscopic coefficient}}{.68}$$

Total organic matter was determined by computing the percentage of weight lost when oven-dry soil was heated in an electric furnace.

EXPERIMENTAL RESULTS

The successional development of plant cover results in definite modifications of the habitat. Each stage of plant growth produces a greater quantity of vegetation than the preceding one. This quantitative increase in amount of vegetation is chiefly a result of increase in height which is possible because of the greater depth and fertility of soil resulting from the disintegration of the underlying rock which, in this experiment, was sandstone.

PERCENTAGE OF PLANT COVER

In the early stages of succession (the lichen and moss stages) the percentage of plant cover may be high (table 1) because there are no tall plants to shade the substratum. After the advent of higher plants in the xerophytic herb and grass stage the dense, but low cover of lichens and mosses is shaded out. From the moss stage to the end or climax, there is a gradual increase in percentage of plant cover as shown in table 1. Height of plants and resulting total weight of plant cover increases from the first to the final stage of the succession. Stages of the xerosere from the initial lichen stage to the post-climax stage (linden-maple association) are shown in figure 1. The crustose lichen stage is important in producing the first thin layer of soil (1-2 millimeters in depth). The quantity of soil of this stage is so small that it was not studied. In plates I and II are shown views representing the gradual change of plant cover as it occurs in nature.

DEPTH OF SOIL

Depth of soil gradually increases from the earlier to the later stages (table 1). The first two stages have no root development but beyond this stage the gradual increase in quantity and depth of roots is one of the chief factors in the increase in soil depth. The presence of these roots also

contributes to the holding of the soil which is an important property of plant covers in the prevention of erosion. Height of plants and resulting total weight of the plant cover increases from the first to the final stage because of the shading out of lower by higher plants.

The quantity of duff resulting from dead plant parts increases from earlier to later stages because of the gradual increase in the total plant cover. Duff is effective in preventing erosion through the protection to the soil surface from the force of rain, and through the effect on texture and structure of the soil to which it is added. Fertility from this decomposition contributes to a gradual increase in quantity of plant cover from the initial to the climax stage of the succession. This increase in the density of the plant cover contributes to its erosion-prevention capacity by breaking rain drops into spray and by means of the soil holding capacity of roots. The depth of soil increases more rapidly in the later stages because the rate of loss by erosion is greatly reduced and the rate of increase in soil depth is accelerated by the addition of larger quantities of duff and by deeper penetration of roots.

WATER-HOLDING CAPACITY OF THE SOIL

The addition to the soil of plant parts which become decomposed, increases the water-holding capacity of the soil. This is illustrated in table 2 by the increase from a water-holding capacity of 24.5 per cent in scraped sandstone, to one of 45 per cent or greater for soil from the same kind of sandstone in which plants are growing. The percentage of water held by the soil of the lichen stage is very high because it was impossible to separate the lichens from the samples studied. The relatively high water-holding capacity of the moss and fern stages may be attributed to the high proportion of dead plant material in the soil. In each succeeding stage above the moss stage there seems to be a consistent increase in water-holding capacity for the upper decimeter of soil. However, the difference is so small that any important increase in water-holding capacity in the higher stages of succession, insofar as it affects erosion control, results from an increase in soil depth rather than from an increase in quantity of water held per unit of soil. With the increase in soil depth an added burden is placed on the vegetation, i. e. to hold the soil against erosion; but this increase in depth is accompanied by an increase in the volume of vegetation which in turn results in greater erosion prevention capacity.

The field method of determining the water-holding capacity of undisturbed cores of soil gives more accurate results for the actual water-holding capacity in the field than does the laboratory method. These results, presented in table 2, show less difference in water-holding capacity of the different stages than do the results of the laboratory method, and more clearly emphasize the fact that the increased depth of soil in the higher stages accounts for the greater water-holding capacity of the site. In tests for the moss stages, the moss plants were left in place on the soil in the cylinder and in the higher stages, the duff was left in place.

WILTING COEFFICIENT

Measurements of water-holding capacity should be expressed in terms of available water if the relation of the habitat to the plant cover is

to be determined. The results reported in table 2 show that the growth of plants on the substratum increases the percentage of water in the soil at wilting (non-available water) as well as the water-holding capacity of the soil. However, the increase in water-holding capacity is so much greater than the increase in non-available water that the difference between the two shows an increase in the available water induced by the presence of plants. The available water in the scraped sandstone in the saturated condition is only about 24 per cent; while it is about 35 per cent for the seven to ten centimeter level of the xerophytic shrub stage, the lowest amount of available water in any level of the sites occupied by plants. The highest percentage of available water is over 87 in the thin soil of the foliose lichen stage, in which the plant bodies of the lichens are included.

TOTAL ORGANIC MATTER

The columns of table 2 for the water-holding capacity and for the organic matter present show a significant positive correlation between the percentage of total organic matter present and the percentage of water held by the soil, although there are to be noted a few exceptions where there is an important difference in organic matter in two soils which are approximately equal in water-holding capacity. In these instances there must be a difference in size of particles.

The five successive stages, in which the plants can be separated easily from the soil, seem to be divided as to percentage of organic matter into two groups: the xerophytic grass and herb and the xerophytic shrub in one group; and the mesophytic shrub, the oak-hickory and the linden-maple in the other group. This difference is probably attributable to the addition each year of greater quantities of dead plant parts from plant cover to the soil of the stages in the second group, for there was a marked break between the two groups in total mass of vegetation and in percentage of cover (table 1). In the latter group the soil is usually covered by a layer of undecomposed or partially decomposed plant parts, varying from three to fifteen centimeters in depth compared to a layer in the former group varying from one to three centimeters.

In the sandy soils studied, the decomposed organic matter acted as an absorbent of water which is largely available to the plant. Because of this fact, the water-holding capacity of the light soil is much increased without materially increasing the quantity of non-available water.

SUMMARY

Quantitative investigation was made of habitat changes accompanying the development of successive stages of xerosere on sandstone ledges in The Ledges State Park in central Iowa.

The area covered by plants in the lichen and moss stages of the xerosere varies from one to eighty per cent of the whole.

The percentage of area covered gradually increases from about ten per cent in the xerophytic herb and grass stage to ninety-six per cent in the climax maple-linden stage.

The vegetation of successive stages is progressively higher in growth and the depth of soil is progressively greater.

TABLE 1. *Height of vegetation and percentage of cover of eight stage sof xerosere*

Stage of the xerosere	Foliose lichens	Reindeer moss (lichen)	Moss species	Xerophytic grass and herbs	Xerophytic shrubs	Mesophytic shrubs	Oak hickory	Linden maple
Height of vegetation	1-4 mm.	2-4 cm.	2-5 cm.	.5-30 cm.	2-140 cm.	5-250 cm.	5 cm. 50 cm. 15 m.	5 cm. 50 cm. 18 m.
Percentage of area covered	10 80	75	78	10.5	11.5	43	90	96
Depth of soil	1-3 mm.	2-12 cm.	3-12 cm.	5-18 cm.	1-25 cm.	2-40+ cm.	20-200+ cm.	50-400+ cm.

Vegetation of the lichen and moss type is more effective, per unit of plant material, in increasing the water-holding capacity of the soil of their habitat, than vegetation of higher plants.

Any increase in the total water-holding capacity or in the quantity of available water in the soil of the higher stages of the xerosere over that of the lower, appears to be attributable to increase in depth of soil rather than to any other measurable soil characteristic.

Examination of several sites representing each stage studied shows that under average conditions the vegetation of each stage with the possible exception of the xerophytic grass and herb stage, has sufficient erosion-prevention capacity to control erosion on 45 per cent slopes.

LINDEN-MAPLE
Tilia americana   Acer nigrum
↑
OAK-HICKORY
*Quercus alba   Quercus maxima   Quercus macrocarpa
Carya ovata   Carya cordiformis*
↑
MESOPHYTIC SHRUBS
*Corylus americana   Zanthoxylum americanum
Celastrus scandens   Ribes gracile
Viburnum affine*
↑
XEROPHYTIC SHRUBS
*Rhus glabra   Ceanothus ovatus   Amorpha canescens
Rosa pratincola   Celastrus scandens*
↑
XEROPHYTIC HERBS AND GRASSES
*Antennaria plataginifolia   Aristida sp.   Carex sp.
Thalictrum dioicum*

FERNS
*Camptosorus rhizophyllus
Polypodium virginianum*

MOSS
*Polytrichum juniperianum   Catharina undulata
Bartramia pomiformis*
↑
FOLIOSE LICHENS
Physcia sp.   Cladonia sp.
↑
CRUSTOSE LICHENS
Lecidia sp.   Coniocybe sp.

Fig. 1. Stage of the xerosere with the dominant species of each stage.

TABLE 2. *Modification of soil characteristics in nine successful stages*

Stage	Depth of soil measured	Hygroscopic coefficient percentage	Non-available water percentage	Organic matter percentage	Water-holding capacity Lab. method percentage	Ave. all depths Lab. method percentage	Water-holding capacity field method cm. per cu. dm.
Scraped sandstone	1-5 mm.	.905	1.33	.386	24.6	24.56	223
Foliose lichens	1-2 mm.	1.937	2.85	36.068	90.7	90.66	450
Moss, thin soil	1-5 mm.	4.645	6.83	8.437	77.3	77.27	350
Moss, species	1-3 cm.	3.350	4.93	6.614	78.3	61.38	350
„ „	4-6 cm.	3.378	4.97	3.739	59.7		
„ „	7-10 cm.	2.277	3.35	2.798	46.1		
Reindeer moss (lichen)	1-3 cm.	2.833	4.17	5.224	69.7	57.78	355
Reindeer moss „	4-6 cm.	2.498	3.67	3.314	53.9		
„ „	7-10 cm.	2.168	3.19	3.232	49.7		
Walking-fern	1-4 cm.	8.239	12.13	19.580	108.10	108.10	
Xerophytic grasses and herbs	1-3 cm.	2.903	4.27	5.739	57.6	47.93	412
„ „	4-6 cm.	1.620	2.38	2.832	46.8		
„ „	7-10 cm.	1.517	2.23	1.873	39.5		
Xerophytic shrubs	1-3 cm.	3.059	4.50	5.762	52.7	45.39	352
„ „	4-6 cm.	2.785	4.03	3.981	43.4		
„ „	7-10 cm.	2.798	4.12	4.020	39.0		
Mesophytic shrubs	1-3 cm.	5.545	8.15	9.551	78.2	59.60	375
„ „	4-6 cm.	5.138	7.55	7.985	55.8		
„ „	7-10 cm.	3.451	5.08	5.410	44.7		
Oak-Hickory	1-3 cm.	6.118	8.99	10.471	77.0	59.99	387
„ „	4-6 cm.	4.262	6.83	5.921	55.0		
„ „	7-10 cm.	3.271	4.71	4.666	48.0		
Linden-maple	1-3 cm.	4.823	7.09	7.586	68.0	55.86	372
„ „	4-6 cm.	4.496	6.67	5.242	61.2		
„ „	7-10 cm.	4.208	6.19	8.686	48.4		

PLATE I

Fig. 1. A sandstone ledge showing average growth of foliose lichens on a surface cov-
ered with crustose lichens.

Fig. 2. A close view of a square decimeter of dense foliose lichens.

Fig. 3. Walking fern invading a site occupied by moss which is invading a lichen area.

PLATE I
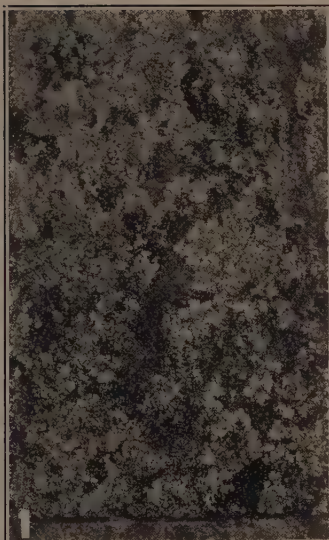
PLATE II

Fig. 1. Ground cover of the oak-hickory stage.

Fig. 2. The xerophytic shrub stage. *Rhus glabra*, smooth sumach, is the dominant.

PLATE II
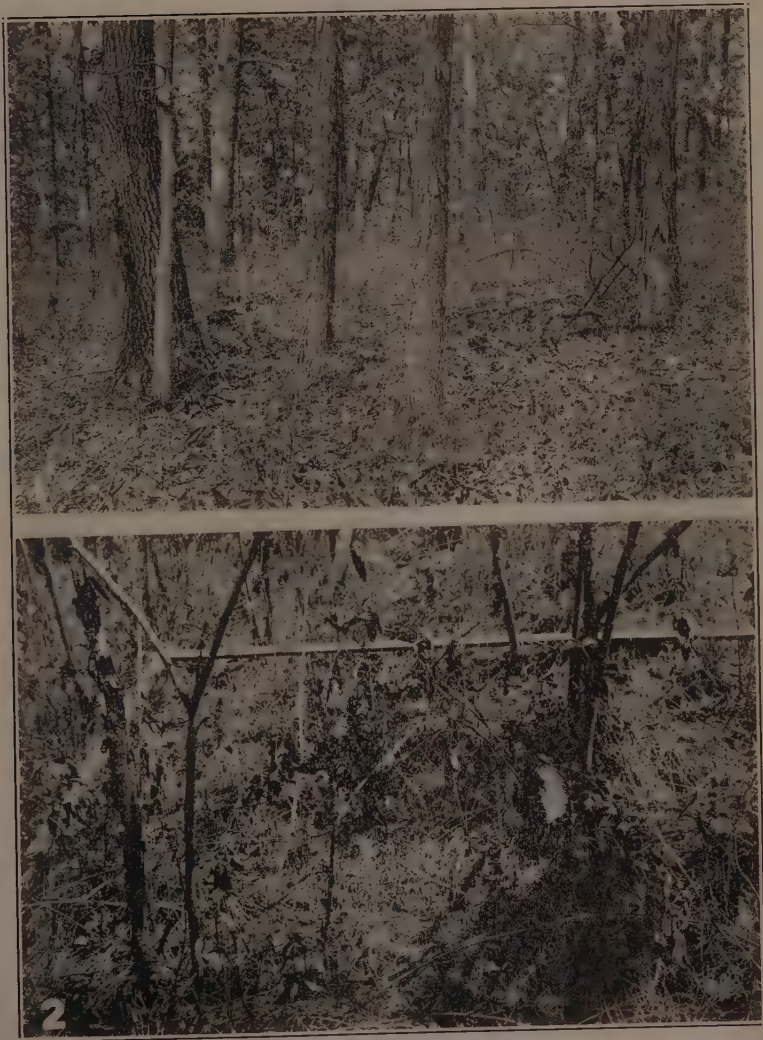
EFFECT OF SPECIES OF GRASSES AND LEGUMES SOWN AND TREATMENT UPON THE POPULATION OF M E A D O W S AND PASTURES [1]

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Cultivated species of legumes and grasses differ markedly in their response to differences in climate, soil, treatment and association with other species. This study was made in order to determine the manner in which cultivated legumes and grasses respond to treatment under meadow and pasture conditions on two soil types on the Iowa Agricultural Experiment Station farms at Ames. Since Kentucky blue grass—the dominant species in these studies—is also dominant in most pastures of Iowa, it is believed that the results here reported may be applicable to most parts of the state.

METHODS OF EXPERIMENTATION

Eight legumes and 16 grasses were planted in 1928 in pure seedings and 12 mixture combinations containing from 2 to 16 species. Rates of seeding used were mainly those recommended in the literature and corrected to conform with laboratory germination of the seed.

Triplicate plantings were made in each of three series. Series I was planted on the Iowa State College dairy farm on Clarion sandy loam soil. This series was pastured with light to moderate severity, annually from 1930 to 1933 inclusive and closely grazed throughout the season of 1934. Two hay crops were removed in 1929.

Series II and III were planted on O'Neill sandy loam soil, and hay crops have been removed annually beginning in 1929. Two crops of hay each year have been removed from series II and five crops from series III.

Population trends are being measured by density-list, permanent, meter quadrat readings of basal cover for which there are five quadrats in each mixture plat of series I, and three in each of series II and III and one quadrat in each of the pure seeding plats. The number of quadrat replications is in order of sizes of plats. For the pasture, series I, quadrat readings have been taken annually except in 1934. For the meadow, readings were obtained annually from 1929 to '32 inclusive for series II and to 1933 inclusive for series III. Final readings for all series are to be taken in 1935.

Estimates of populations of dominant species were made in early November, 1934, in order to give the reader a general idea of the present stands of the plats. Because these observations were taken in late fall when conditions for identification from vegetative growth were not satisfactory the estimates are subject to correction in May and June, 1935, when final quadrat readings will be made. Since estimates are based on appearance from a distance of five feet the estimated cover percentages are much higher than actual basal cover readings would show.

Legumes tested in pure seedlings with most of them also included in the mixtures are alfalfa, *Medicago sativa* L., medium red clover, *Trifolium pratense* L., wild red clover, *T. pratense* L. (Obtained from Europe), white clover, *T. repens* L., wild white clover, *T. repens* L. (Obtained from Europe), ladino clover, *T. repens latum* McC., Alsike clover, *T. hybridum* L. and biennial yellow sweet clover, *Melilotus officinalis* L.

Grasses tested included Kentucky blue, *Poa pratensis* L., Canada blue, *P. compressa* L., rough-stalked meadow, *P. trivialis* L., meadow fescue, *Festuca elatior* L., sheep's fescue, *F. ovina* L., slender wheat, *Agropyron tenerum* Vasey, crested wheat, *A. cristatum* L., perennial rye, *Lolium perenne* L., orchard, *Dactylis glomerata* L., tall oat, *Arrhenatherum elatius* L., brome, *Bromus inermis* Leyss, red top, *Agrostis alba* L., crested dog's-tail, *Cynosurus cristatus* L., sweet vernal, *Anthoxanthum odoratum* L., timothy *Phleum pratense* L. and reed canary, *Phalaris arundinacea* L.

The 12 mixtures designated by A to L, inclusive, follow in this paragraph with the number after the species indicating the rate of seeding per acre. It may be observed that trifolium clovers and timothy were basic ingredients of each of the mixtures: A—medium red 7, timothy 8; B—alsike 2, medium red 4, timothy 4, Kentucky blue 7; C—white 2, alsike 2, medium red 4, timothy 4, brome 7; D—white 2, alsike 2, medium red 4, timothy 4, Kentucky blue 7; E—ladino 2, alsike 2, medium red 4, timothy 4, Kentucky blue 7; F—wild white 2, alsike 2, medium red 4, timothy 4, Kentucky blue 7; G—white 1.5, alsike 1.5, medium red 3, alfalfa 3, timothy 2, Kentucky blue 3, brome 3; H—white 1.5, alsike 1.5, medium red 3, biennial yellow sweet 3, timothy 2, Kentucky blue 3, reed canary 3; I—white 1.5, alsike 1.5, medium red 3, timothy 2, Kentucky blue 3, orchard 3, red top 2; J—white 1.5, alsike 1.5, medium red 3, timothy 2, Kentucky blue 3, orchard 2, meadow fescue 2, brome 2; K—timothy 3, Kentucky blue 5, orchard 3, meadow fescue 3, brome 3; L—white 0.5, alsike 0.5, medium red 1.5, timothy 3, Kentucky blue 3, orchard 1, meadow fescue 1, brome 3, reed canary 1, slender wheat 1, perennial rye 1, tall oat 1, rough-stalked meadow 1, crested wheat 1, sheep's fescue 2.

RESULTS

SURVIVAL OF SPECIES IN PURE SEEDINGS

Twelve of the 16 grasses sown have survived in pure seedings, but 11 of them are gradually being supplanted by Kentucky blue grass. The species of grass still existent are Kentucky blue, Canada blue, sheep's fescue, red top, slender wheat, orchard, brome, timothy, reed canary, meadow fescue, crested wheat and tall oat grass. The percentages of basal cover when quadrat readings were last taken in 1932 and '33 and the apparent cover when estimates were made in November, 1934, are shown in table 1. The 12 surviving species indicated in the table are arranged in order of their percentages of basal cover of quadrat readings taken in the pasture series in 1933, and it may be observed that data obtained for the other series and in other years agree fairly well.

TABLE 1. *Densities of surviving species of grasses sown in pure seedings in 1928 together with those of Kentucky blue grass, the predominating immigrant*

	Percentage cover					
	Pasture	Meadow		Pasture	Meadow	
	Series I	Series II 2-crops	Series III 5-crops	Series I	Series II 2-crops	Series III 5-crops
		Quadrats			Estimates	
Species	1933	1932	1932	1934	1934	1934
Kentucky blue	36	43	49	85	90	95
Canada blue	36	25	24	35	4	5
Kentucky blue	8	18	14	35	86	90
Sheep's fescue	34	46	42	20	55	55
Kentucky blue	8	0	0	20	4	4
Red top	28	21	9	20	48	12
Kentucky blue	4	3	4	20	6	64
Slender wheat	26	24	13	6	25	14
Kentucky blue	T	T	1	44	40	56
Orchard	20	24	22	26	69	67
Kentucky blue	2	T	T	4	1	8
Brome	18	17	14	26	72	54
Kentucky blue	2	3	5	4	8	36
Timothy	16	33	21	4	41	9
Kentucky blue	16	T	1	36	9	81
Reed Canary	16	19	19	25	32	9
Kentucky blue	2	12	8	25	48	81
Meadow fescue	14	18	4	3	36	8
Kentucky blue	8	2	4	17	4	64
Crested wheat	10	22	17	38	76	64
Kentucky blue	14	1	1	12	2	17
Tall oat	6	13	6	T	51	49
Kentucky blue	8	T	T	20	1	9

The predominant immigrant in each of the series is Kentucky blue grass, other grasses and weeds being relatively unimportant. While percentages of basal cover for quadrat readings are small, space not occupied by the encroaching Kentucky blue grass is largely vacant. Usually the space occupied by weeds and other grasses besides Kentucky blue has been

less than 3 per cent, and this same condition has existed through 1934. Exceptions have been meadow fescue and tall oat grasses which have not been effective against competition of the winter annual brome grasses, *Bromus secalinus* L. and *B. Japonicus* Thunb. when these species flourish in May.

Orchard and sheep's fescue grasses have withstood invasion of Kentucky blue grass well, as compared with the others. Brome and crested wheat grasses rank next in this respect, but they have been supplanted markedly by Kentucky blue where five crops of hay have been removed per season in the meadow.

It is apparent that severe treatment favors the encroachment of Kentucky blue grass. In the meadow all of the other species have maintained better stands and have been invaded less by Kentucky blue grass with the removal of but two crops per season than of five. Tall oat grass, however, has maintained good stands in both series II and III of the meadow but is practically extinct in the pasture. This is thought to be mainly due to reseeding in the meadow where all 2- and 5-crop plats are side by side throughout. The grass has had little opportunity to reseed itself in the pasture.

In contrast with the other species Kentucky blue grass has maintained itself in almost pure condition in the pasture and both of the meadow series except for slight encroachment of dandelion, *Traxacum officinale* Weber.

The four extinct grass species are crested dog's-tail, sweet vernal, perennial rye and rough-stalked meadow. Stands were not obtained of crested dog's-tail and sweet vernal grasses, of the latter probably because of weakly germinating seed. Perennial rye grass was killed by freezing the first winter as it has been in other tests at the Iowa Station. Rough-stalked meadow grass perished during the extremely dry and hot summer of 1930.

All of the legume species except alfalfa and sweet clover, were killed either during the severe drouth of the summer of 1930 or by freezing during the winter of 1930-'31. Sweet clover died at the end of its biennial period in 1929. Excellent stands of the trifolium clovers persisted into the second crop year of 1930. Only scattered seedlings of any of the legumes have appeared since the initial seeding, and these have been largely of white clover.

Alfalfa has persisted in each of the series but is nearly extinct in the 5-crop meadow. A fair stand is still apparent in the 2-crop meadow with considerable still remaining in the pasture. In another test at the Iowa Agricultural Experiment Station the removal of four alfalfa crops the first-crop year resulted in a large reduction in stand by the second-crop year. Kentucky blue grass has encroached to predominant proportions in each of the series, but the turf is by far the most dense in the 5-crop meadow.

POPULATION OF MIXED SEEDINGS

As may be noted from table 2, Kentucky blue grass is strongly dominant on all plats in which seed of this species was included in the mixtures, and the cover is much thicker than for seedings made without Kentucky blue grass. In fact, in the pasture, grazed within an inch of the ground in 1934, plats seeded with clover-timothy or clover-timothy-brome, the only

TABLE 2. *Densities of surviving species of legumes and grasses sown in mixtures in 1928*

Mixture	Species*	Rate seeded (lbs. per acre)	Percentage cover					
			Pasture Series I	Meadow Series II 2-crops	Meadow Series III 5-crops	Pasture Series I	Meadow Series II 2-crops	Meadow Series III 5-crops
			1933	Quadrats 1932	1932	1934	Estimates 1934	1934
A	Timothy	8	26	32	21	20	40	36
	Kentucky blue	0	4	T	2	50	4	14
B	Timothy	4	2	7	T	1	8	5
	Kentucky blue	7	30	37	54	92	81	90
C	Timothy	4	8	18	9	14	12	9
	Brome	7	14	8	10	35	48	27
	Kentucky blue	0	4	1	5	1	20	54
D	Timothy	4	2	7	1	2	4	1
	Kentucky blue	7	30	35	51	90	81	94
G	Alfalfa	3	2	10	6	T	4	T
	Timothy	2	2	3	1	2	12	5
	Kentucky blue	3	24	15	39	75	32	78
	Brome	3	4	9	3	15	32	9
H	Timothy	2	2	4	T	1	4	1
	Kentucky blue	3	22	28	53	82	64	85
	Reed canary	3	6	7	1	10	12	4
I	Timothy	2	2	3	T	1	4	1
	Kentucky blue	3	20	19	34	81	73	86
	Orchard	3	6	13	7	11	12	5
	Red top	2	2	0	0	1	T	T
J	Timothy	2	2	2	T	2	3	1
	Kentucky blue	3	18	27	40	82	64	85
	Orchard	2	6	5	6	3	12	2
	Brome	2	4	3	T	3	12	7
K	Timothy	3	2	2	1	2	3	1
	Kentucky blue	5	18	30	36	82	64	85
	Orchard	3	4	6	6	3	20	4
	Brome	3	4	2	1	3	4	5
L	Timothy	3	2	4	1	1	2	1
	Kentucky blue	3	22	23	38	80	62	85
	Orchard	1	2	1	2	3	12	3
	Brome	3	4	4	2	3	12	3
	Reed canary	1	2	T	1	3	3	3

* Only those species which have more than one per cent of cover are given. Complete lists of ingredients and rates of seeding are given under "Methods of Experimentation."

mixtures in which Kentucky blue grass was not included, appear rather barren as compared with the much more abundant cover of plats in which Kentucky blue grass was included in the mixtures. Also, Kentucky blue grass has immigrated surprisingly slowly into plats where seed was not included in the mixtures, and it is believed that in farm fields the volunteering of Kentucky blue grass would be even slower. On the whole, other species have resisted the competition of Kentucky blue grass much more effectively in the 2-crop meadow than in the 5-crop meadow and the pasture. As in the case of the pure seedings, Kentucky blue grass has been practically the only invading species. Clovers disappeared in 1930 as in the pure seedings and only occasional plants, principally of white clover, have been found since.

Principal data for 10 of the 12 mixtures are given in table 2. Mixtures E and F are omitted because they are similar to D except that ladino and wild white clover, respectively, were used instead of common white. Only those species which have more than one per cent of cover are shown in the table.

Mixture A, consisting of medium red clover and timothy, is commonly used on Iowa farms. Since mixture B is essentially similar, except that Kentucky blue grass was added, a comparison of the two is interesting. Where Kentucky blue grass was added it practically has exterminated the timothy, and this has taken place much more rapidly with more severe treatment of the pasture and 5-crop meadow as compared with the much less severe treatment of the 2-crop meadow. Moreover, the total cover is much more dense where Kentucky blue grass was included in the mixture. Where Kentucky blue grass was not added it has invaded the timothy slowly, the most rapid increase occurring in 1934 in the pasture which was closely grazed during this year. It also gained rapidly in the 5-crop meadow in 1934 and markedly less rapidly in the 2-crop meadow. Apparently where Kentucky blue grass was not included it has been increasing in logarithmic proportions in the pasture and 5-crop meadow.

Mixture D is practically a repetition of mixture B, the only difference being in the species of clovers and rates of their inclusion in the two mixtures. It may be observed that the data for the two mixtures agree closely, both for the quadrat readings and the estimates.

Mixtures C and D are counterparts except that brome grass was used in C and Kentucky blue in D. It may be noted from the data for both quadrat readings and estimates of table 2 that the total cover for the brome grass mixture in the pasture is much thinner, and that timothy has competed much more successfully in the brome grass mixture than in the Kentucky blue in all series. It is apparent that Kentucky blue grass also, although not seeded, has invaded the brome grass mixture, but this, as in the clover-timothy mixture, has been done slowly. The blue grass has invaded and displaced brome and timothy most in the 5-crop meadow and has invaded the pasture but little, possibly because of larger plots and chance location. In the pasture where the invasion of Kentucky blue grass is estimated at only one per cent the total cover of timothy, brome and Kentucky blue is only 50 per cent as compared with 90 per cent for the 5-crop meadow where the invasion of Kentucky blue is estimated at 54 per cent. Apparently Kentucky blue grass occupies considerable cover-free space in a timothy-brome grass sod before crowding them to extinction.

In addition to the basic ingredients of clover and timothy, mixture G included alfalfa, Kentucky blue grass, and brome. Alfalfa, timothy, and brome are gradually being displaced by Kentucky blue grass in the order given, and this is more pronounced in the 5- than in the 2-crop meadow. The total cover is fairly similar to that of mixtures B and D which contained only Kentucky blue grass in addition to the basic ingredients of clover and timothy.

For mixture H, made up of Kentucky blue and reed canary grasses in addition to clover and timothy, somewhat less of reed canary grass remains than of brome grass in mixture G. For mixture I containing Kentucky blue grass, orchard and red top in addition to the basic clover and timothy ingredients red top was practically extinct when quadrat readings were last taken in 1932 and '33, while orchard grass has maintained its stand about like the stand of reed canary grass in mixture H. For J in which orchard, brome, and meadow fescue were added to the clover-timothy-Kentucky blue grass mixture, orchard has resisted Kentucky blue grass somewhat more effectively than brome while meadow fescue was practically extinct in 1932-'33. Mixture K is a counterpart of J except that clovers were omitted from K, and practically similar data have been obtained for the two mixtures. Eisele and Aikman [3] in reporting early results for these two mixtures stated that growth of the grasses was stimulated by the clovers which is a general observation. For mixtures H, I, J and K blue grass has sodded more thickly in the 5-crop meadow than in the 2-crop, and estimates indicate that stands of the other grasses are thinner in the 5-crop meadow.

Quadrat readings for Mixture L indicate that of the 12 grass ingredients 7 were practically extinct in 1932 and '33. Species still in existence in appreciable quantities were Kentucky blue, timothy, orchard, brome, and reed canary grasses. Those which were extinct or essentially so were meadow fescue, sheep's fescue, slender wheat, crested wheat, perennial rye, tall oat and rough-stalked meadow. When quadrat readings were last taken in 1932 and '33 Kentucky blue grass comprised 69 per cent of the cover in the pasture, 72 per cent in the 2-crop meadow and 86 per cent in the 5-crop meadow, while the four other existent species, timothy, orchard, brome and reed canary grasses were about equal in the 5-crop meadow, timothy and brome were superior in the 5-crop meadow, while brome was superior to the other three in the pasture. The greatest percentage of cover, however, of any of these minor species in any location, was 4 so that it is doubtful if differences are significant.

Quadrat readings indicate that when a meadow is to be used later as a permanent pasture as little as 3 pounds of Kentucky blue grass seed per acre on fertile soil should provide a fair cover of this species after 4 or 5 years, but that a 7-pound rate probably would be better. The percentages of Kentucky blue grass cover with 3-pound rates, as an average for the three series, varied between 24 and 28 per cent for the four mixtures, G, I, J and L. (Averages for series I, II and III are not shown in table 2 but can be caculated readily from the data given.) The competing species and their rates of seeding varied, of course, but apparently reacted similarly on the Kentucky blue grass. Total rates of seeding competing grass

[3] Eisele, H. F., and J. M. Aikman. 1933. The development and survival of species and varieties in planted pastures. Ecology 14:123–135.

species were 5, 7, 8 and 16 pounds per acre for mixtures G, I, J and L, respectively. For the 7-pound rates of seeding Kentucky blue grass with timothy the only competing grass seeded at 4 pounds per acre, the Kentucky blue grass cover varied between 39 and 41 per cent for mixtures B, D, E and F as averages of the quadrat readings for series I, II and III. While these percentages of cover seem low it should be emphasized that there appeared to be full stands of good cover when viewed from a distance of 5 feet for plats in which Kentucky blue grass was included in the mixtures.

SUMMARY

In 1928, 8 legumes and 16 grasses were planted in pure seedings and in 12 mixture combinations containing from 2 to 16 species. Triplicate plantings were made in each of three series. Series I was pastured from 1930 to '34, inclusive. Hay crops have been removed annually from series II and III, two crops from the former and five from the latter. Population changes are being measured with density-list, permanent, meter quadrat readings. Final readings are to be made in 1935.

Of 16 grass species sown in pure seedings the 12 still existent are Kentucky blue, Canada blue, sheep's fescue, red top, slender wheat, orchard, brome, timothy, reed canary, meadow fescue, crested wheat and tall oat.

Practically, alfalfa is the only legume species which has persisted. The others planted were biennial yellow sweet clover and the trifolium clovers—medium red, wild red, alsike, white, wild white and ladino.

The predominant immigrant in each of the series is Kentucky blue grass which has been gradually replacing the other species. Other grasses and weeds have been unimportant except that meadow fescue and tall oat grasses have not been effective against competition of annual brome grasses.

It is plainly apparent from series II and III that severe treatment favors encroachment of Kentucky blue grass into pure seedings, and in mixtures other species have resisted the competition of Kentucky blue grass much more effectively in the 2-crop meadow than in the 5-crop meadow and the pasture.

Kentucky blue grass is strongly dominant on all plats in which seed of this species was included in the mixtures, and the cover is much thicker than for seedings made without Kentucky blue grass. Also, Kentucky blue grass has immigrated surprisingly slowly into plats where seed was not included in the mixtures.

In mixture L, made up of 3 trifolium clovers and 12 grass species, all species are practically extinct except Kentucky blue, timothy, orchard, brome, and reed canary grasses. Grass species which are extinct or essentially so are meadow fescue, sheep's fescue, slender wheat, crested wheat, perennial rye, tall oat and rough-stalked meadow.

MEASUREMENT OF RUN-OFF AS INFLUENCED BY PLANT COVER DENSITY[1]

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Little is known concerning the influence of plant cover density on run-off and erosional losses in southern Iowa, where farming practice has greatly reduced the area of undisturbed forest litter and the original prairie vegetation, both of which were efficient in maintaining the soil in a stable and water-receptive state. Today temporary crop covers and poorly sodded pasture covers, whose culture and long overgrazing have exposed much of the soil surface to the direct action of rain and flowing waters, have replaced these effective erosion preventing plant covers. In 1932 an attempt was made to measure the value of different vegetative covers in preventing run-off losses. The observations and data obtained are recorded, following a description of the methods and plots.

METHOD

In May, 1932, 15 plots, size 1/200 acre, on Lindley and Shelby loams, were established on farms in the vicinity of Indianola, Warren County, Iowa. The protection which the plot covers afforded the surface soil was determined from run-off measurements made after each storm. The ground area protected by the plant cover on each plot was estimated as plant cover density. The slope of these plots varied from eight to 14 per cent.

Measurements of run-off were obtained by locating modified Bates and Zeasman[2] "erosion traps," 6.6 feet in width, at the lower end of the 33-foot plot sites. These traps consisted of a slanting galvanized iron trough and a removable six- or 10-gallon bucket, both of which were covered with heavy tarpaper and lath in order that rain falling directly on the trap would not be recorded as run-off. Each trough was made of one piece of sheet iron so that a shoulder or lip four inches wide extended above the slanting trough its entire width, 6.6 feet. After a rectangular shaped pocket had been dug at the lower edge of the plot, this shoulder or lip was carefully worked up hill into the soil of the plot at a depth sufficient to prevent the loosening of the soil directly on the shoulder from that of the plot proper. The shoulder served as a bond between the trap and the plot. It is thought that this arrangement permitted the movement of run-off water from the plot into the slanting trough without any appreciable loss. Following each rain the trap covers were removed, the traps examined, the bucket contents measured and the bucket emptied, the trough cleaned, and the trap assembled and recovered. At times the bucket was not large enough to take care of the run-off. In such a case,

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[1] Abstract.
[2] Bates, C. G., and O. R. Zeasman. Soil erosion, a local and national problem. Wis. Agr. Exp. Sta., Res. Bul. 99. August, 1930.

the bucket's capacity, 10 gallons in all cases of overflow, was used in calculations of total run-off or run-off as the percentage of rainfall.

The upper limit of each plot was marked by a shallow ditch and a mound built to prevent the movement of run-off water from the surrounding field on the plot. Several of the plots were observed during rains to determine whether or not the ditches were of sufficient protection. The movement of run-off to and from the plot along the sides was unhindered; however, this error was reduced to a minimum by selecting a site which sloped in only one direction, namely toward the trap. By mid-summer it was possible to evaluate run-off as percentage of rainfall, for a modified Snowden type rain gauge had been placed at each plot.

After the traps were placed and in working order, estimates were made of the protection to the soil surface afforded by the plant cover against the direct impact of the rain. The estimates, termed the density of cover on the ground, represent the proportion of ground actually covered by plant material, living or dead. This may be expressed in tenths, or, as in this study, in units; thus 10/10 or 10 indicates full coverage of the soil. If in a corn field only 1/10 of the ground surface is protected by plant bodies at the ground, the ground cover density is represented by the figure one.

It was soon recognized that certain planted crops as soybeans, sweet clover and sorghum had a heavy top growth but offered only scanty protection at the ground level. Thus the protecton which these covers afforded the soil against the direct impact of rain is restricted largely to that given by the leaves and stems. For this reason, estimates of crown density for each plot were made on the same basis as was used in estimating ground cover density. To facilitate density estimates, pins were set at five-link intervals around the periphery of the plot to be studied. By connecting opposite pins with string, the plot was divided into 20 squares, five links on a side. For each square, estimates of the ground and crown cover densities were made and recorded on a chart representing to scale the subdivided plot. The figures used subsequently in discussion of cover density represent the modal values of the estimates for the 20 subdivisions of each plot.

DESCRIPTION OF PLOTS

The sample plots selected for the run-off studies were designated by the dominant vegetation in the field or, in the case of certain crops, by the name of the crop which was harvested during the season. These plots were located on four distinct areas in the county. Since the plots of an area are grouped in the discussion, they are listed here by areas.

Hazel brush, bluegrass plot of area No. 1.

This area, situated on Shelby loam, had a slope of 11 per cent and a southeast exposure. Evidently the land had never been plowed though certain stumps indicated that there had once been a sparse forest growth above the shrubs. The area was pastured by hogs, cattle and horses during the experiment.

Weeds in old field plot of area No. 1

The soil type, slope and aspect of this plot was comparable to the hazel brush bluegrass area. The two areas were only about 30 feet apart.

It was evident that this area had been under cultivation, and after being seeded to sweet clover had been pastured for the preceding four years. It was pastured throughout the experiment.

Second growth oak-hickory plot of area No. 1.

This plot was located on Shelby loam, slope nine per cent, with a northerly aspect. The plot showed little evidence of recent disturbance. The area was pastured lightly by heifers during the summer.

Sudan grass plot of area No. 1.

This plot was on Shelby loam of 11 per cent slope with a westerly exposure. The field had produced a poor crop of corn the previous year. This area had been subjected to heavy erosional losses, and considerable areas of yellow sub-soil were exposed. The grain was inadvertently destroyed by cattle in mid-August. The four plots in this area were within three-eights of a mile of one another.

Wheat drilled across plot of area No. 2

The Shelby loam soil of this plot sloped to the east at the rate of 14 per cent. The wheat had been cut before the plot had been established. Here the grain had been sown when the drills were moving at right angles to the slope. The field was not pastured.

Wheat drilled down plot of area No. 2.

This area in the same field as the previous plot was also on Shelby loam having a slope of 10 per cent in an easterly direction. The grain had been drilled in rows parallel to the slope at this point in the field. The previous crop had been oats.

Timothy and clover plot of area No. 2.

The soil here was Shelby loam of 13 per cent, having a northerly aspect. This plot was situated in a field which had been seeded down one year previous. The top soil had been eroded to some extent, leaving at the surface numerous stone fragments. One cutting was made on the plot. There was no current grazing.

Corn plot of area No. 2.

This area of Shelby loam on a westerly slope of 11 per cent had yielded two cuttings of timothy and red clover the year before. After the seed had been planted the field had been dragged with a harrow which moved up and down the slope in the vicinity of the plot.

Sorghum plot of area No. 3.

This plot was also located on Shelby loam of 10 per cent slope in a southeasterly direction. The field in which the plot was located had been in alfalfa the four preceding years.

Oats plot of area No. 3.

The plot was located on Shelby loam, 12 per cent slope, easterly exposure. The crop had been cut before the trap was placed. The stand was thin and the grain so short that some of it was left standing on the plot. The previous crop had been corn.

Alfalfa plot of area No. 4.

The soil type of this plot was Lindley loam with a slope of 10 per cent to the southeast. The field had been in alfalfa the three previous years. Three cuttings had been taken from the field the year before and one during the growing season in question. Hogs were turned into the field in mid-summer.

Sweet clover plot of area No. 4.

This plot was located on Lindley loam soil with an eight per cent slope to the west. This area was in a badly eroded field which had not been cropped since 1930.

Ragweed plot of area No. 4.

This area on Lindley loam of 13 per cent slope with exposure to the east had not been cropped for six years. The area was pastured by hogs and horses during the experiment. The area had suffered from serious past erosion.

Barley plot of area No. 4.

This plot was on Lindley loam, nine per cent slope, northeast exposure. The crop was removed after the trap had been in operation. The plot was not pastured during the study.

Soybean plot of area No. 4.

The Lindley loam soil of this plot was badly eroded and low in organic matter. The slope was 10.5 per cent with a northerly exposure. The previous crop had been corn. The beans had been drilled parallel to the slope. There was no grazing during the period in which the measurements were made.

EXPERIMENTAL DATA

The plots designated by their plant covers and arranged in order of decreasing ground and crown cover density, together with the rainfall and the run-off as percentage of rainfall for four late summer storms, are listed in table 1. This table indicates that the protection given the ground and estimated as ground cover density, varied from a minimum of one in the case of sorghum, corn, sweet clover, soybeans, and ragweed covers, to a maximum of 10 for the second growth oak-hickory and the hazel brush-bluegrass covers. The plots from which barley and oats had been harvested had an estimated ground cover density of two and three, respectively. The two plots from which wheat had been harvested had a ground cover density of 2.5, as was the case of the timothy and clover plot cover. The ground cover of the alfalfa plot was composed of some straw and considerable moss tufts which largely accounted for the ground cover estimate of four. The weeds in the old field plot had a ground cover density of four. This estimate was only exceeded by the ground covers on the hazel brush and oak-hickory plots. Three of the covers studied were comparable in that they had been allowed because of low fertility to revert from cultivated land to pasture land. The ragweed and the sweet clover covers both had ground density values of one, while the weeds in the old field ground cover was estimated at four.

TABLE 1. *Plant cover density estimates, detailed rainfall and plot run-off records following four rains on two types of soil in Warren County, Iowa*

		Cover density		Per- cent- age slope	Measurements			
	Plant cover	Ground	Crown		Aug. 24–25	Aug. 31	Sept. 8–9	Sept. 11–12
Soil Type—Shelby Loam	Second growth oak– hickory	10	8	9				
	Inches rainfall				0.78	0.38	0.47	0.70
	percentage run-off				0.28	0.19	0.03	0.37
	Hazel brush-bluegrass	10	8	12				
	Inches rainfall				0.78	0.47	0.42	0.70
	Percentage run-off				0.09	0.00	0.44	0.10
	Wheat drilled across (timothy, clover and weeds)	2.5	6	14				
	Inches rainfall				0.53	0.77	0.18	0.37
	Percentage run-off				0.00	0.57	0.00	0.00
	Wheat drilled down (timothy, clover and weeds)	2.5	6	10				
	Inches rainfall				0.50	0.73	0.20	0.37
	Percentage run-off				2.95	1.00	trace	0.59
	Weeds in old field	4	4	12				
	Inches rainfall				0.78	0.47	0.42	0.70
	Percentage run-off				1.23	0.16	8.77	2.95
	Oats (weeds)							
	Inches rainfall				1.09	0.53	0.01	0.80
	Percentage run-off				6.75*	12.50	0.00	9.21*
	Timothy and clover	2.5	4	13				
	Inches rainfall				0.43	0.40	0.88	0.36
	Percentage run-off				0.17	0.00	0.62	0.00
	Sorghum	1	4	10				
	Inches rainfall				0.50	0.94	0.49	0.015
	Percentage run-off				14.72*	7.80*	9.02	0.00
	Corn	1	1	11				
	Inches rainfall				0.53	0.54	0.24	0.38
	Percentage run-off				3.61	13.60*	0.92	2.90
Soil Type—Lindley Loam	Alfalfa	3	8	10				
	Inches rainfall				No	0.58	0.19	1.01
	Percentage run-off				data[1]	0.00	0.00	4.45
	Sweet clover	1	9	8				
	Inches rainfall				0.89	0.60	0.20	0.96
	Percentage run-off				0.00	0.24	0.00	0.77
	Barley (weeds)	2	5	9				
	Inches rainfall				0.84	0.60	0.20	1.02
	Percentage runoff				3.50	0.12	0.00	1.37
	Ragweed (small)	1	2	13				
	Inches rainfall				0.90	0.66	0.13	1.01
	Percentage run-off				2.70	0.93	0.00	7.29*
	Soybeans	1	0	10.5				
	Inches rainfall				0.95	0.58	0.19	1.01
	Percentage run-off				1.00[2]	1.14	0.00	7.29*

* Trap overflowed. Percentage determined on basis of run-off in bucket, 10 gallons.

[1] Hogs turned into field and upset trap.

[2] Crop removed August 23.

It is evident that for the late summer period, the crown density estimates ranged from zero in the case of the soybean plot, which had been previously cut, to nine for the volunteer sweet clover cover. The corn cover density was one, that for the ragweed cover was two. An estimate of four was applied to the crown cover density of the hazel brush-bluegrass, timothy and clover, weeds in old field, oats (weeds), and sorghum covers. In the case of the plot from which barley had been removed, the annual weeds had developed a crown density of five. A crown density estimate of six was applied to the covers in the wheat plots. The estimate of crown cover density for the second growth oak-hickory and the alfalfa covers was eight.

Referring to table 1, the total rainfall for the storm period August 20 to August 25 varied from 0.50 inch at the wheat drilled down plot and at the sorghum plot to 1.09 inch at the oats plot. An outstanding variation of rainfall occurred during the storm period, September 8 to September 9, when the rainfall at the timothy and clover plots was 0.88 inch while that at the wheat field plots, about 0.4 mile away, was 0.18 inch and 0.20 inch. Similar variations in rainfall occurred during the other storms as indicated in the table.

Because of the great variation in quantity of rainfall incident on the plots during a given storm as indicated by the rain gauge records, a comparison of the run-off data collected as related to the influence of plant cover density must be restricted to neighboring plots. Consequently the plots in the same area will be grouped in the discussion.

The total rainfall, 0.78 inch, at the hazel brush-bluegrass plot (ground cover density of 10, and crown density of 8) for the storm of August 24 and 25 gave a measurable run-off of 0.09 per cent of the rainfall. The run-off from the oak-hickory cover (ground cover 10, crown cover 8) was three times that from the hazel brush-bluegrass, while the run-off collected in the trap at the weeds in old field plot (cover density 4 at ground and in crowns) was more than 13 times as much. At another farm the timothy and clover plot (ground cover 2.5, crown cover 4) yielded the least measurable run-off, 0.17 per cent, of the 0.43 inch rain. This was exceeded 17 times by the run-off collected at the wheat drilled down plot (cover density 2.5 at ground and 6 in crowns), and 21 times by the run-off at the trap in corn (ground cover 1, crown cover 1). There was no run-off in the wheat drilled across. Both the oats plot (ground cover 3, crown cover 4), receiving 1.09 inch, and the sorghum plot (ground cover 1, crown cover 4), receiving 0.50 inch, yielded run-off in excess of 10 gallons as indicated by the asterisks in the table. Of the plots on the Lindley loam which gave measurable run-off, the plot in the soybean field (ground cover 1, crown cover 0), crop removed, gave 1.0 per cent run-off, ragweed (ground cover 1, crown cover 2) 2.70 per cent run-off, and 3.50 per cent loss for the plot in the barley (ground cover 2, crown cover 5) field. The bucket at the sweet clover trap was empty (ground cover 1, crown cover 9) though the rain gauge yielded 0.89 inch rainfall.

For the storm of August 31 the least measurable run-off was recorded at the weeds in old field plot. Here only 0.16 per cent of the 0.47 inch rain has been lost through run-off. The adjacent hazel brush-bluegrass trap was dry, while the oak-hickory trap yielded run-off equivalent to 0.19 per cent of the 0.38 inch rain. The wheat plots having received about the

same rainfall allowed run-off of 0.57 and 1.00 per cent of rainfall for the wheat drilled across and drilled down the slope, respectively. No run-off occurred from the timothy and clover cover. The corn trap was over-flowed from the 0.54 inch rain which fell on the plot. The plots, sorghum and oats, receiving 0.53 inch and 0.94 inch rainfall yielded run-off losses of 12.5 per cent in the case of the oats and of more than 10 gallons on the sorghum plot. The run-off losses from the plots on the Lindley loam in-crease from nothing at the alfalfa plot to 1.14 per cent of the rainfall at the soybean plot.

The data for the storm of September 8 and 9 indicate great variation in total rainfall and suggest considerable variation in intensity of the showers. Although the gauge at the oak-hickory plot indicated more total rainfall than at the hazel brush-bluegrass plot, the run-off at the latter area exceeded that from the oak-hickory by more than 14 times. The run-off of 8.77 per cent of rainfall at the weeds in old field plot exceeded that from the hazel brush-bluegrass plot by nearly 20 times. The only other traps containing run-off water were at the timothy and clover plot with 0.62 per cent, the corn plot with 0.92 per cent and the sorghum plot 9.02 per cent. The plots having received 0.20 inch rain or less did not lose any moisture through run-off.

During the storm period of September 11 and 12 the hazel brush-bluegrass plot yielded only 0.10 per cent of the 0.70 inch rain as measur-able run-off at the trap. This was exceeded nearly four times by run-off in the trap under the oak-hickory cover, and nearly 30 times by the run-off collected from the weeds in old field plot. The rain at the wheat plots resulted in a 0.59 per cent run-off in the case of wheat drilled down the slope; however, no run-off was collected from the trap located where the wheat had been drilled across the slope. The corn plot showed a 2.9 per cent rainfall loss during the same period. The rain of 0.80 inch at the oats plot overflowed the trap. The sorghum plot one-half mile away received only 0.015 inch of rain during this storm. For the plots on the Lindley loam the sweet clover plot receiving 0.96 inch rain yielded 0.77 per cent of this as run-off to the trap. The run-off from the alfalfa plot was ap-proximately six times this amount, while the barley plot trap record was only twice that of sweet clover run-off. The run-off from soybeans and ragweed plots exceeded the trap capacity.

The cover densities representative of the protection afforded the soil during a greater part of the summer, the number of storms for which run-off records are available, the total plot run-off and the mean plot run-off are given in table 2. The arrangement of the data is according to increas-ing mean run-off. For the last eight plots, accurate total run-off records are not available, since with the equipment used, volumes greater than 10 gallons could not be measured.

The weeds in old field (ground cover 4, crown cover 4) yielded run-off which exceeded trap capacity on three occasions. The traps in the sudan grass (ground cover 4, crown cover 2), ragweed (ground cover 2, crown cover 1), and wheat drilled down (ground cover 6, crown cover 2.5), were overflowed four different times. Two plot traps failed during five storms to accommodate all the run-off. These were the traps in the corn (ground cover 1, crown cover 1), and oats (ground cover 4, crown cover 3) fields. The traps in soybeans (ground cover 5, crown cover 1) and

TABLE 2. *Summary of plant cover density and run-off data obtained for the season from 1/200 acre field plots*

Plant cover of plot	Cover density		Num- ber storms	Total run-off gallons	Mean run-off gallons
	Ground	Crown			
Hazel brush-bluegrass	10	8	11	5.1	0.46
Second growth oak-hickory	10	8	11	8.4	0.76
Sweet clover	1	9	10	9.0	0.90
Wheat, drilled across (timothy, clover, weeds)	2.5	6	6	8.5	1.41
Barley (weeds)	2	5	11	19.91	1.81
Timothy and clover	2.5	4	8	17.45	2.18
Alfalfa	3	8	7	24.46	3.49
Weeds in old field	4	4	11	*46.70	4.24
Sudan grass	2	4	11	*51.05	4.64
Ragweed	1	2	11	*53.90	4.90
Wheat drilled down (timothy clover, weeds)	2.5	6	8	*42.80	5.45
Corn	1	1	11	*61.70	5.52
Soybeans	1	5	11	*64.80	5.89
Sorghum	1	4	13	*98.50	7.58
Oats	3	4	8	*61.00	7.62

* Run-off exceeded the trap capacity. The figure given is based on the measurable run-off in the trap, thus is lower than the actual.

sorghum (ground cover 4, crown cover 1) were overflowed seven and nine times, respectively.

The two plots, hazel brush-bluegrass (ground cover 10, crown cover 8) and weeds in old field (ground cover 4, crown cover 4), subjected to similar conditions of rainfall and use during the study are best suited for direct comparison. The run-off collected from the trap beneath the hazel brush-bluegrass cover amounted to 5.1 gallons for the season, while the measurable run-off collected under the weeds in old field plant cover amounted to 46.70 gallons, or nearly 10 times as much.

The records of the two plots in the wheat interplanted to timothy and clover having comparable cover density indicate a run-off loss of 1.4 gallons per storm in the case of the plot located so that the drill rows contoured the plot; while the average run-off from the plot, drill rows down the slope, amounted to 5.45 gallons. This suggests that other factors than plant cover density may influence the amount of run-off lost from a given cover.

From table 2 it is evident that of the seven plots whose run-off never exceeded the trap capacity only one plot had been plowed during the previous 12-month period. Of the plots which yielded run-off in quantities greater than 10 gallons sometime during the study only two plots, the weeds in old field plot and the ragweed plot, had not been plowed during the previous 12-month period.

SUMMARY

The study reported herein was undertaken to determine the relative effectiveness of various plant covers in preventing run-off losses on soil typical of some of the rough agricultural land in southern Iowa. This was accomplished by measuring the run-off collected in erosion traps from 1/200 acre plots located in farm fields on four areas of Warren County, Iowa. These records were supplemented by estimates of the soil surface area which was protected against the direct impact of the rain by the existing plant cover. The mean plot run-off for the season was hazel brush-bluegrass 0.46 gallon, second growth oak-hickory 0.76 gallon, sweet clover 0.90 gallon, wheat drilled across 1.41 gallons, barley (weeds) 1.81 gallons, timothy and clover 2.18 gallons, alfalfa 3.49 gallons, weeds in old field 4.24 gallons, sudan grass 4.64 gallons, ragweed 4.90 gallons, wheat drilled down 5.45 gallons, corn 5.52 gallons, soybeans 5.89 gallons, sorghum 7.58 gallons, oats 7.62 gallons. The barley (weeds) plot was the only area which, having been plowed in the spring, did not yield run-off in amounts greater than the capacity of the trap.

The density estimates indicate a difference of one to 10 in the protection given to the plot soil by the various plant covers. The annual crops offered only slight protection to the soil as indicated by the estimates, corn 1-1, soybeans 1-5, sorghum 1-4, sudan grass 2-4. Such undisturbed covers as second growth oak-hickory and hazel brush-bluegrass had fairly dense covers which were estimated as 10-8. These last mentioned plot covers yielded the least volume of run-off water.

The plots having covers of wheat stubble planted to timothy and clover which had cover density estimates of ground 2.5, crown 6, differed in that the direction of the old drill rows aided the movement of run-off water in one case and retarded such action on the other plot. The average run-off from the wheat plot drilled down grade was 5.45 gallons, while from the nearby plot with the plants drilled at right angles to the grade, even with increased slope, the run-off averaged 1.41 gallons per storm.

Although there is a tendency for run-off to vary inversely with plant cover density, the fact that the field plots were located in four different areas in the county on two distinct soil types admits a lack of uniformity in cultural treatment and soil conditions. These factors were not measured in this study.

3. Symposium: Applied Botanical Research on Maize

The Iowa Corn Research Institute has been established through cooperation of the United States Department of Agriculture and various other agencies with the Iowa Agricultural Experiment Station. Its purpose is further to encourage and coordinate all maize research in Iowa, and to increase its usefulness, recognition, and financial support. A full account of the Institute, including its purpose and scope, is available in the form of a brochure of the Agricultural Experiment Station.

CONTENTS

APPLIED BOTANICAL RESEARCH ON MAIZE

R. E. BUCHANAN

From the Iowa Agricultural Experiment Station

It is well known that the maize plant is the chief source of Iowa's eminence in agriculture. Maize flourishes in our rich soil and is adapted to our climate, yielding normally from four to five hundred million bushels of a concentrated food admirably adapted for conversion into potential energy through biological and chemical agencies. No further justification is needed for the researches relating to the numerous aspects of maize culture and its utilization. The concern to us as a research station staff is, rather, to consider whether our present efforts and the moneys expended are yielding the largest returns in the form of new knowledge and its application to production and utilization. Our station, like many others, is organized into sections, sub-sections, and projects with specialists as leaders, cooperators, and coordinators, each focusing his or her attention upon certain specific objectives. In some instances research projects are rooted in institutions supported by other funds, either federal or industrial; and in many other cases, as in the industries, researches on maize or its products have no relation, either cooperative or coordinate, with Iowa State College. It is believed that the Iowa Corn Research Institute, created as an integral part of the Iowa Agricultural Experiment Station, may be able to contribute a unifying medium for its own varied researches relating to maize, and likewise for those researches that are cooperative with other institutions. It is hoped, too, that industrial agencies having need for research may find in the Institute a more suitable place for the solution of their problems than in their own independent laboratories.

The Institute should serve also to band together a group of specialists interested in the solution of problems relating to maize, to provide a unifying medium, and to supply a place where problems relating to industry may be solved, and to function as a clearing house for researches that have been, or are being, made. We are about to witness in these meetings a demonstration of these last named functions through its sponsoring of a symposium in a field of research. It is hoped that in the future, groups of specialists may be brought together for the consideration of phases of maize research other than those which engage our attention today, such as chemical, industrial, and economic aspects.

Such gatherings as this will not only bring to light new facts and principles, but also acquaint the different workers with the progress and problems that prevail in related fields. The border line fields and the general trend of the advance of our knowledge should, through such meetings, come to stand out in sharper relief. In addition, such symposia as this should pave the way to new researches borne out of the efforts and thought of many workers in the field.

Time will not permit me on this occasion to dwell at length upon the general scope of the Institute. The purpose and organization of the Iowa

Corn Research Institute of the Iowa Agricultural Experiment Station has been set forth in a brochure already available for general distribution. Today, at this the first program of the Institute, we have chosen to direct our attention to a symposium entitled: "Applied Botanical Research of Maize."

RESISTANCE AND SUSCEPTIBILITY OF CORN STRAINS TO SECOND BROOD CHINCH BUGS

J. R. Holbert, W. P. Flint, J. H. Bigger, and G. H. Dungan

From the Bureau of Plant Industry, United States Department of Agriculture, Illinois State Natural History Survey and Illinois Agricultural Experiment Station

The devastating action of chinch bugs on the corn crop in central Illinois during the past two years has resulted in disappointing yields of inferior quality in the heavily infested areas (Pl. I). But a more important effect of this insect pest has been the hardship and dicouragement that have come to the many individual growers whose corn crop has been almost completely wiped out. Although properly constructed and well maintained barriers are effective in combating the advance of the first brood of bugs out of small grain into the corn, and that in itself is highly worthwhile, no known measures are available for preventing adult bugs of the first brood from flying into the corn fields in mid-summer and depositing eggs from which hatch the second brood that feeds on the crop until it matures or is killed. The development and use of strains of corn comparatively resistant to the action of the second brood of bugs appears to be the most practical and effective method of guarding against unbearable losses to large groups of individual growers. The development of such resistant strains will allow the grower to assure himself an approximately predetermined production without over-planting to balance possible losses.

In the southern part of the central corn belt of Illinois and further south, where the frequently recurring cycles of chinch bugs have been an important factor in limiting corn production for almost a century, natural selection has resulted in the development of a few open-pollinated strains that have considerable chinch bug resistance. Flint and Hackleman in a publication issued in 1923[1] called attention to the resistance of Champion White Pearl (sometimes called "Democrat"), of certain strains of Golden Beauty, and of some other open-pollinated varieties to damage by second brood bugs. The merits of these open-pollinated strains, particularly Champion White Pearl and Waddell Golden Beauty, under heavy chinch bug infestation in the region in which they are adapted (south of the 40th parallel) have been demonstrated by repeated tests.

The situation in the greater part of central Illinois is entirely different. All generally grown commercial varieties that have been tested have proved to be more or less susceptible. Even in parts of central Illinois where chinch bugs have been an important consideration 7 years out of 10 for the last 25 years, the local varieties that have been tested have proved to be comparatively susceptible. And for this section varieties like Champion White Pearl and Golden Beauty require a longer season than usually is available.

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[1] W. P. Flint and J. C. Hackleman, Corn Varieties for Chinch Bug Infested Areas, Illinois Bulletin No. 243, 1923.

This present chinch bug outbreak has been very disturbing to those having the responsibility for the cooperative corn improvement program in the state. Some inbreds believed to have an established place in the contemplated commercial production of double crosses that had stood high in yield tests for several years were injured to such an extent that little or no seed was harvested. Fortunately, however, not all the so-called good inbreds that have been developed as a result of the cooperative efforts of the workers connected with the State Experiment Stations in the corn belt and with the United States Department of Agriculture have been susceptible. A few lines have been found, at least one from each of the group of lines exchanged with workers in the other corn belt states, that have stood up reasonably well as inbreds under heavy second brood chinch bug infestation. These lines have transmitted resistance to hybrids, as determined by the performance of top crosses and three-way and double crosses involving these lines when grown under heavy chinch bug infestation[2].

The scatter diagram in figure 1 was prepared according to the top cross method suggested by Jenkins[3] from data taken in 1933 from a large yield experiment throughout which the infestation was unusually uniform. The high degree of correlation ($r = 0.84$) between the actual and
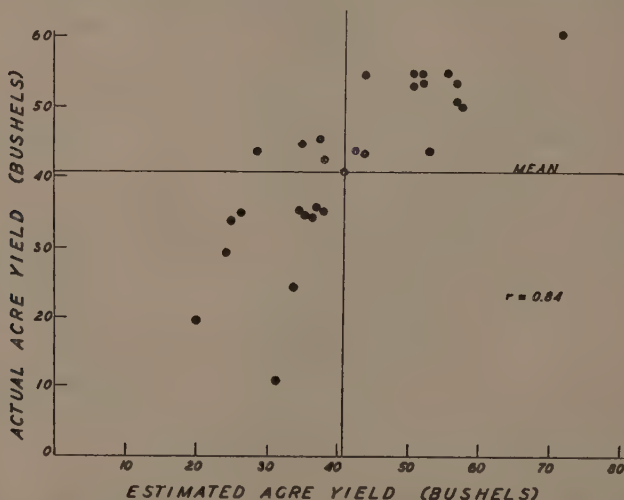
Fig. 1. Scatter diagram of the actual acre yield of 29 double and three-way crosses under heavy second brood chinch bug infestation and the estimates of their yields obtained by calculating the mean of the values for the top crosses of the parental lines when grown under heavy chinch bug infestation, Bloomington, Illinois, 1933.

[2] J. R. Holbert, W. P. Flint and J. H. Bigger, Chinch Bug Resistance in Corn—An Inherited Character, Journal of Economic Entomology, February, 1934.

[3] Merle T. Jenkins, Methods of Estimating the Performance of Double Crosses in Corn, Journal American Society of Agronomy, March, 1934.

estimated yields under heavy second brood bug infestation would seem to suggest that this method also has merit for estimating the probable yields of double crosses under heavy chinch bug infestation when the behavior of the parental lines in top crosses under heavy bug infestation is known (Pl. II).

STUDIES IN 1934

In addition to the nine cooperative experimental yield tests located in different corn growing sections of the state in which varieties and both experimental and commercial hybrids were entered, thirty cooperative field tests were conducted in 10 central Illinois counties. Data from the field tests only are presented in this paper. These field tests, for the most part, consisted of 2-, 4-, and 6-row, machine-planted plots extending across the fields, special care being taken to select fields as uniform as possible in productivity and general topography. In one field the plots were 20 rows wide. In another field in which the corn was hand-planted, the individual plots were 10 hills wide and 15 hills long. The intensity of chinch bug infestation usually has varied considerably throughout the field. For this reason long plots extending across the field have had a distinct advantage in that the portions to be harvested could be selected in the part of the field in which the infestation was reasonably uniform and of sufficient intensity to furnish a basis for determining comparative chinch bug resistance. All fields were inspected for intensity and uniformity of infestation the latter part of August.

Plots only two rows wide have not proved as satisfactory for studying comparative chinch bug resistance as have plots 4 and 6 rows wide. In the 2-row plots strains adjacent to very susceptible strains frequently have been subjected to an infestation of double intensity, or more, compared to the average of the field due to the migration of the bugs away from the dead plants of the susceptible strains to the green plants of adjacent resistant strains. For the most part only the center rows of the wider plots were harvested.

Three groups of experimental hybrids were selected by the top cross method (fig. 1) for planting in the field tests, one group predicted to be susceptible, one resistant and another intermediate in resistance. The cooperating farmer's own seed was used for one of the varieties. These tests also included six commercial hybrids, other varieties commonly grown in that vicinity, and a few top crosses.

The plots were harvested the last week in October and the first week in November by representatives of the cooperating institutions, who also were responsible for taking all the data.

Data are presented in table 1 giving yields of two standards chinch bug-resistant varieties, Champion White Pearl and Waddell Golden Beauty, and one double cross (Illinois Hybrid 391). Both resistant varieties stood up better under heavy chinch bug infestation than the local varieties and also yielded more sound corn in the Sangamon county plot. Hybrid 391 gave a substantial increase in yield over the resistant varieties in both fields.

The Swanson field in Knox County was unusual in that one end of the field had only a very light infestation while the center of the field had a heavy late infestation. On the part of the field where the bugs were not a

TABLE 1. *Percentage standing plants, total and sound corn yields from local open-pollinated varieties, two chinch bug-resistant varieties (Champion White Pearl and Waddell Golden Beauty) and one double-cross (Illinois Hybrid 391) ranking high in chinch bug resistance, all grown under heavy second brood chinch bug infestation, Sangamon and Mason Counties, Illinois, 1934*

		Acre yield	
Kind of corn	Percentage plants standing	Total bu.	Sound (Not conspicuously damaged by bugs or ear rots) bu.
SANGAMON COUNTY			
Local varieties	51.0	22.8	9.0
Chinch bug-resistant varieties—			
Champion White Pearl	85.3	21.1	16.2
Waddell Golden Beauty	77.1	23.5	16.0
Illinois Hybrid 391	82.4	32.8	22.6
MASON COUNTY			
Local varieties	86.0	33.0	29.6
Chinch bug-resistant varieties—			
Champion White Pearl	92.6	32.7	30.9
Waddell Golden Beauty	95.8	31.6	29.4
Illinois Hybrid 391	97.8	40.3	37.6

factor influencing yield, the bug-susceptible and bug-resistant hybrids were about equal and both significantly better than the local variety, the increases being 14.2 bushels and 11.7 bushels, respectively (table 2). But on the part of the field where the late infestation was very heavy the bug-susceptible hybrids were little better than the local varieties, while the chinch bug-resistant hybrids in this part of the field gave an increase of 14.6 bushels per acre. Perhaps the most noticeable difference between the local varieties and the bug-resistant hybrids was the difference in lodging, the percentages of standing plants being 14.0 and 44.4 per cent, respectively (Pl. III).

Again, in the McKeighan field in Knox County, where the infestation was light, the suspectible and resistant hybrids were about equal in yield and both above the local variety, the increases being 7.0 bushels and 6.3 bushels, respectively (table 2). On the Leigh field in the same section of Knox County the same bug-susceptible group proved to be somewhat inferior to the local variety. The bug-resistant hybrids under the heavy infestation in this field, however, yielded 20.4 bushels more than the bug-susceptible hybrids and 16.2 bushels more than the local variety.

PLATE I

ABOVE—Typical damage from a heavy infestation of second brood chinch bugs on some of the less productive soil in McLean County, Illinois, 1934.

BELOW—Typical damage from a heavy infestation of second brood bugs on very productive soil near Bloomington, Illinois, 1934.

PLATE II

THE GROWING OF TOP CROSSES UNDER HEAVY SECOND BROOD CHINCH BUG IN-
FESTATION HAS PROVED TO BE A VERY RELIABLE METHOD FOR DETERMINING
THE REACTION OF INBRED LINES TO CHINCH BUGS IN HYBRID COMBINATION.

Two Iowa top crosses, Iowa I163 x Krug (left) and Iowa I205 x Krug (right), under
early and heavy second brood chinch bug infestation (above) and under very
light and late infestations (below), McLean County, Illinois, 1934.

PLATE III

THE USE OF CHINCH BUG-RESISTANT HYBRIDS APPEARS TO BE THE MOST EFFECTIVE
WAY TO COMBAT SECOND BROOD CHINCH BUGS.

A local open-pollinated variety (left) and a chinch bug-resistant hybrid (right)
grown under late and heavy second brood bug infestation (above) and under
very late and light bug infestation in the same field (Swanson Field, Table 2),
near Galesburg, Illinois, 1934. The corn in front of the baskets is rotted or badly
damaged, the corn in the baskets is sound.

PLATE IV

Local variety (left) and chinch bug-resistant hybrid (right) growing under early and heavy second brood chinch bug infestation in McLean County, Illinois, 1934. The basket on the left of each pair contains the sound corn and the one on the right the damaged corn. One hundred hills were harvested in each case.

The data in table 3 summarizes the results from 20 cooperative field tests with heavy second brood bug infestation in eight central Illinois counties. The sound yields for the most part represented corn that was

TABLE 2. *Comparison of local open-pollinated varieties, chinch bug-susceptible and chinch bug-resistant hybrids, each growing under very light infestation with second brood bugs and under heavy infestation with second brood bugs, Knox County, Illinois, 1934*

| Kind of corn | Percentage plants standing pct. | Acre yield | | Increase (+) or decrease (—) in yield of sound corn of hybrids compared with local open-pollinated varieties bu. |
		Total bu.	Sound (Not conspicuously damaged by bugs or ear rot) bu.	
SWANSON FIELD, LATE AND LIGHT INFESTATION				
Local open-pollinated varieties	66.1	77.5	71.7	
Chinch bug-susceptible hybrids	71.3	89.6	85.9	+14.2
Chinch bug-resistant hybrids	88.6	85.5	83.4	+11.7
SWANSON FIELD, LATE AND HEAVY INFESTATION				
Local open-pollinated varieties	14.0	63.5	53.0	
Chinch bug-susceptible hybrids	7.7	68.0	55.5	+ 2.5
Chinch bug-resistant hybrids	44.4	73.4	67.6	+14.6
McKEIGHAN FIELD, LATE AND LIGHT INFESTATION				
Local open-pollinated varieties	78.9	69.9	66.2	
Chinch bug-susceptible hybrids	74.3	75.2	73.2	+ 7.0
Chinch bug-resistant hybrids	96.9	74.4	72.5	+ 6.3
LEIGH FIELD, EARLY AND HEAVY INFESTATION				
Local open-pollinated varieties	41.8	43.2	35.7	
Chinch bug-susceptible hybrids	28.4	42.2	31.5	— 4.2
Chinch bug-resistant hybrids	67.6	57.4	51.9	+16.2

not on the ground. Perhaps much of the badly damaged corn on down stalks would have been left in the field by farmers. In this connection it is of considerable interest that the average sound yield of the local varieties was only about two bushels above the estimated average state yield of 20.5 bushels. The chinch bug-susceptible hybrids yielded less than the open-pollinated varieties, and those hybrids intermediate in resistance yielded only 4.1 bushels above the varieties. The chinch bug-resistance varieties, however, yielded 12.6 bushels or 55.3 per cent better than the average of the local varieties.

DISCUSSION

The development and use of strains of corn more resistant to damage from second brood bug infestation appears to be the most logical and feas-

TABLE 3. *Summarized data from 20 cooperative field tests in 8 central Illinois counties comparing local open-pollinated varieties and hybrids differing in chinch bug resistance, all fields heavily infested with second brood bugs, 1934*

| | | Acre yield | |
| | | Total | Sound (Not conspicuously damaged by bugs or ear rots) |
Kind of corn	Percentage plants standing pct.	bu.	bu.
Local varieties	46.9	35.4	22.8
Chinch bug-susceptible hybrids	42.1	27.3	13.3
Hybrids intermediate in resistance	58.6	39.7	26.9
Chinch bug-resistant hybrids	65.2	46.3	35.4

ible method of attacking the problem. The fact that chinch bugs are recurring with increasing frequency in the corn belt area of central Illinois suggests the desirability of giving attention to chinch bug resistance in connection with the general corn improvement program. New hybrid strains of corn developed for sections in which chinch bug outbreaks are frequent must possess, in addition to other recognized requirements such as high yield of sound corn and wind resistance, more than average resistance to second brood chinch bugs if their place in the corn production of such sections is to be of permanent value. Continued cooperation of workers interested in the control of this insect pest and workers throughout the corn belt who are breeding corn should make the solution of this difficult problem possible.

These results, to date, suggest the possibility of producing hybrids that are not only outstanding in yield and quality of grain in years when chinch bugs are not present, but that also possess a high degree of resistance to damage from second brood chinch bug attack, thus making such strains of very great value in years of heavy chinch bug outbreaks (Pl. IV).

LOSS MUTATIONS IN MAIZE[1]

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The apparent gene mutations induced by X-rays are probably in many instances short deficiencies without lethal effect on the haploid gametophyte. The evidence for this inference has been summarized elsewhere[2].

A deficiency or other intra-chromosomal alteration transmitted without loss through both male and female gametophytes cannot be distinguished genetically from a gene mutation, unless it includes the loci of two or more known genes separable by crossing over; also, cytological demonstration of deficiency becomes increasingly difficult as the region affected becomes smaller. It may, therefore, be impossible to demonstrate deficiency by either genetic or cytological means, in specific instances in which the deficiency is small enough to be transmitted without loss through both male and female gametophytes.

Many of the induced variations are partially haplo-viable and are thus intermediate in genetic behavior between the typical deficiencies and the typical gene mutations. In some of these, deficiencies large enough for cytological identification in pachytene are found. Several cases of this sort are described in the present paper, forming an intergrading series between the typical deficiencies and the typical mutations. In some instances the deficiency is transmitted regularly through female gametophytes and partially through male gametophytes. This indicates that shorter deficiencies, probably not cytologically demonstrable, would in some cases permit fully normal transmission through both male and female gametophytes and would thus simulate the genetic behavior of typical gene mutation.

A method was described for the demonstration of deficiency in cases in which the chromosome segment lost is too short to permit cytological identification. This may be accomplished by the production of shortened deficiencies (with accompanying duplications) from haplo-viable deficiencies of demonstrable extent, through the occurrence of crossing over in regions of non-homologous pairing. Studies made with deficiency 5_1, an internal deficiency including the locus of the gene V_3 in chromosome 5, in which non-homologous pairing is fairly frequent, show that crossing over may occur in regions of non-homologous pairing with the production of viable derivative types.

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[1] Abstract of paper presented in Symposium on "Applied Botanical Research of Maize."

[2] Proc. VI Intern. Cong. Genetics I, pp. 274-294. 1932.

THE EFFECT OF INBREEDING AND OF SELECTION WITHIN INBRED LINES OF MAIZE UPON THE HYBRIDS MADE AFTER SUCCESSIVE GENERATIONS OF SELFING [1]

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The effects of self pollination and of selection upon the resulting inbred lines of corn have been studied in considerable detail in the past. So far as is known to the writer, no very complete study has been made of the performance of crosses made after successive generations of inbreeding for the purpose of studying (1) the effect of inbreeding (i.e. increased homozygosity) and (2) the effect of selection within the parental lines upon hybrids involving them. Richey and Mayer [2] studied crosses made after 2, 3, 4, and 5 generations of selfing and found no outstanding differences between the average yields of the crosses made after the different generations. The crosses after 2 generations of inbreeding were lower yielding than the others. The effect of selection was studied by Richey and Sprague [3] in back-pollinated lines and in crosses made after successive generations of back pollination. Selection apparently was effective in producing yields larger than those theoretically expected without selection.

Inasmuch as the practical use of inbred lines, for the present at least, appears to be in the production of hybrids, it seemed that a more detailed study of hybrids made after successive generations of inbreeding might yield information of value. An experiment was planned, therefore, to determine the effect of inbreeding and of selection (as it had been practiced in the Iowa breeding program) upon crosses made after successive generations of selfing. The basis of the selection has been similar in general to that usually practiced in inbreeding programs. Major emphasis has been placed on the selection among progenies with minor emphasis on plant selection within progenies. Selection has been for yield of sound corn, general plant vigor, desirable plant type and resistance to lodging.

MATERIALS AND METHODS

Remnant seed of all the generations of inbreeding of 28 inbred lines which had been selfed for 8 generations were used, 14 of the lines being from Iodent, a rather closely bred selection of Reid Yellow Dent, and the

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[2] Richey, Frederick D., and L. S. Mayer. The Productiveness of Successive Generations of Self-Fertilized Lines of Corn and of Crosses between Them. U. S. Department of Agr. Bul. 1354, 18 p., illus. 1925.

[3] Richey, Frederick D., and George F. Sprague. Experiments on Hybrid Vigor and Convergent Improvement in Corn. U. S. Dept. Agr. Tech. Bul. 267, 22 p., illus. 1931.

List of the Inbred Lines Included in the Experiments

Iodent Lines	Lancaster Lines

```
        3     2                                   1 1 2 1
I   154–4–2–2–3–1–1–2–1   (A)          L   287–4–2–3–2–2–3–1–2   (A1)
       4 3   2 3   2                                  4 4   3

      1 1 1 1 1                              1     1 1 3
I   163–3–3–2–2–2–3–1–1   (A2)         L   289–4–3–5–2–3–4–1–1   (A1)
                   3                            4 6       2

       1       1                               3   1 3
I   173–3–5–2–1–2–3–2–1   (B)          L   291–1–6–1–3–4–2–1–1
       4   3 2 3     2                          3 6     4   2

      2 3 2   1 1                            1       3
I   182–4–4–4–2–2–3–1–1                L   292–3–1–2–3–1–4–1–1   (A)
            3       3                           3 3 4 2     2

       3   2 1 1 1                           1 3   2   2
I   185–5–1–3–3–2–3–1–1   (B)          L   293–3–4–1–3–1–3–1–1   (A1)
       6           2                            4   2     2

      1 4     1 3                               2   1 2
I   188–2–5–1–1–2–4–1–1   (B)          L   295–2–2–4–1–3–4–2–1
          3 3       3                           3 5   2       3

        2                                      4   2
I   197–1–2–6–4–1–1–1–1   (A1)         L   304–1–1–5–1–3–1–1–1   (A)
       4 3   5 2 4   2                          2 3   2   2   2

          1                                  1   2 1
I   211–4–4–1–1–2–1–2–1   (A)          L   309–1–2–2–2–3–2–1–1
       5 5 4 2   2   3                          2   3 3       3

          1                                      1   1
I   219–3–1–2–2–2–1–1–1                *L  311–4–1–3–2–1–3–3–1
       4 3 6 3   3   2                          5 3 4       2

      1   1 1                                 1   1 2 3 1
I   224–2–2–1–2–2–2–2–1   (A1)         L   317–3–1–2–5–4–2–1–1   (B2)
       3   2     4   2                          3           3

      1   1   1                               1     1 1 1
I   234–2–3–1–4–1–3–2–2                L   320–3–1–5–3–3–2–1–1   (A2)
       5   2   2     3                            5 6         2

      2 1   1 3 1
I   238–3–2–6–2–4–3–1–2                L   324–2–2–1–3–1–1–1–1
       7           3                            3 3 2 4 2 2   2

       3     1 1                              1       1
I   242–2–5–2–1–3–4–2–2   (A2)         L   331–3–1–5–1–2–1–2–1   (A)
       3   3 2       3                            4 7 2   2   2

      1 3 1                                    2     2 2
I   252–2–3–5–2–1–1–1–1                L   337–3–1–2–2–4–3–2–1
       3       2 4   2                            5 3 3       3
```

* Only one progeny in the fifth generation of selfing.

remaining 14 from Lancaster Surecrop, a very broadly bred variety. These represented a random sample of the lines in the two varieties that had survived 8 generations of inbreeding. Seed was taken from the remnants of the ears representing the direct line of descent in the different generations of inbreeding from the first to eighth, inclusive, except the seventh. These remnants represented the progenies in each generation, except the eighth, which had been selected to continue the pedigree. A remnant representing a sister progeny in each generation was taken at random from among those which had been grown and discarded in favor of the selected progeny. The pedigrees of the lines included are recorded below. The first number in each case represents the ear number of the open-pollinated ear from which the line was started. The direct line of descent of the selected progenies is shown in the continuous horizontal pedigree. The pedigrees of the discarded progenies in the different generations which were included for comparison are shown above or below the continuous pedigrees. For example, I 154 is represented in the first inbred generation by progenies 3 and 4. Progeny 3 was discarded and progeny 4 is represented in the second inbred generation by progenies 2 and 4, etc.

Top crosses were used to determine the performance value of the inbred lines in crosses. The remnants were grown in 1931 and as many as possible of the resulting progenies were top crossed on the Krug variety. In crossing, pollen was collected from several plants of an inbred line, usually 10 or 12, mixed and applied by hand to 10 ear shoots of the open-pollinated variety. The season was extremely hot and dry at pollinating time and an unusually poor set of seed resulted. No seed was obtained of many of the combinations and only small amounts of others. Some crossed seed was obtained from the combinations with each inbred line except L 331A which therefore had to be excluded. There was insufficient seed of the inbred lines to permit a repetition and it was decided to proceed with the seed obtained. Some combinations were included which had as few as 3 pollinated ears—representing a sample of only 3 plants from the Krug variety. Most of the combinations, however, were represented by mixed seed from at least 5 ears. It is recognized that the variety was sampled inadequately in some crosses, but it is felt that the averages involving several crosses should be reasonably reliable.

The top crosses were compared in 1932, those with the Iodent lines being grown in one group and those with the Lancaster Surecrop lines in another. Six 1-row plots, 14 hills long, were planted to each cross where seed permitted. The plots were distributed at random within six blocks. Acre yields were determined and data were taken on a number of other characters of the plants and ears.

DATA ON YIELD

The data on yield are more extensive than those on the other characters studied. For this reason they are presented separately. Those on the other characters are grouped and discussed in a later section.

EFFECT OF SELECTION BETWEEN SISTER PROGENIES

The effect of selection between sister progenies upon the acre yields of their hybrids was studied in crosses made after the first to sixth

TABLE 1. *Acre yields in bushels for the Krug top crosses of the selected and discarded sister in the eighth selfed generation (indicated as No. 1 and*

Inbred parent	First			Second			Third ,		
	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.
I 154A	84.5	78.6	5.9	80.3	80.2	0.1	90.0	80.1	9.9
I 163A2	73.8	67.8	11.0	94.8	74.6	20.2	79.0	78.6	.4
I 173B	80.5	57.0	23.5	74.7	....	....	....	....	....
I 182	76.7	86.0	— 9.3	85.6	78.6	7.0	85.9	72.3	13.6
I 185B	63.4	79.1	—15.7	80.1	80.8	— 0.7	81.3	81.6	— .3
I 188B	88.3	77.0	11.3	....	....	....	....	....	....
I 197A1	76.6	69.7	6.9	91.0	70.1	20.9	84.0	84.5	— .5
I 211A	88.4	82.0	6.4	....	....	....	....	....	....
I 219	75.8	61.3	14.5	80.5	....	....	91.1	85.9	5.2
I 224A1	89.9	57.7	32.2	96.8	89.5	7.3	91.5	97.9	— 6.4
I 234	74.2	83.1	— 8.9	87.3	90.7	— 3.4	78.4	91.0	—12.6
I 238	73.4	....	....	86.4	85.7	.7	81.4	79.6	1.8
I 242A2	....	....	....	79.6	....	....	81.2	93.6	—12.4
I 252	95.6	72.0	23.6	88.8	83.3	5.5	96.9	89.0	7.9
Mean differences			8.45			6.40			0.60
σ (Mean Diff.)			4.16			2.94			2.57

generations of inbreeding, inclusive. Selection in the first generation of inbreeding was among selfed progenies descended from the same open-pollinated ear. The data on the crosses after the eighth generation of inbreeding were not used in this study as no selection had been practiced as yet in this generation. Because of the fragmentary nature of the data, it was not possible to use them in a single, complete analysis of variance as had been planned. Instead, the crosses of the selected and discarded sister progenies of each inbred line in each generation of inbreeding were paired and the differences between pairs determined. The data are shown in tables 1 and 2.

The mean differences for the first and second generations of inbreeding in Iodent (table 1) are more than twice their standard errors, indicating that the crosses of the selected progenies yielded significantly more than those of their discarded sibs. The mean differences for the generations of inbreeding after the second in Iodent and for all generations in Lancaster Surecrop (table 2) are too small to be considered significant. All but one of the mean differences are positive, however, and indicate a consistent trend in favor of the crosses of the selected progenies, though the individual means are not large enough to be considered significant as judged individually by their standard errors.

It should be borne in mind that the selection measured in these data is that among plants and related progenies *within* lines as the lines were rapidly becoming homozygous through selfing. The possibilities of differential selection naturally are much less in the later generations of inbreeding and are smaller than would have been the case had the selection been among unrelated lines.

This effect may be shown in the following comparison. The standard error of the acre yields in table 1 is 3.8 bushels and of those in table 2 is 4.2 bushels. Differences between the crosses of sister progenies in table 1 of 10.7 bushels (twice the standard error of the difference) and in

progenies from the first six selfed generations of the Iodent lines and of two sister progenies No. 2) between which no selection had been made as yet

Fourth			Fifth			Sixth			Eighth	
Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	No. 1	No. 2
94.9	100.6	— 5.7	85.8	....	....	81.2	76.1	5.1	79.5	84.3
....	....	....	91.2	....	....	......	......	....	......	....
70.0	69.4	0.6	70.2	....	....	73.3	......	....	68.6	72.9
98.8	94.1	4.7	96.9	90.7	6.2	94.9	87.2	7.7	98.0	103.0
72.7	75.1	— 2.4	70.2	76.4	— 6.2	79.5	73.4	6.1	77.7	....
....	....	....	......	......	....	......	......	....	81.0	73.3
68.9	71.1	— 2.2	92.5	83.7	8.8	79.1	82.2	— 3.1	70.8	80.1
....	....	....	......	......	....	......	......	....	69.8	89.5
97.6	86.9	10.7	......	......	....	80.8	83.2	— 2.4	87.7	84.4
100.8	102.2	— 1.4	102.8	82.1	20.7	......	......	....	91.6	105.5
88.9	87.5	1.4	91.9	87.3	4.6	89.6	93.8	— 4.2	......	89.2
80.3	77.1	3.2	87.8	84.3	3.5	89.1	83.5	5.6	80.1	82.8
82.7	....	....	78.1	83.9	— 5.8	83.4	80.3	3.1	78.9	87.7
90.6	88.9	1.7	73.6	88.9	—15.3	92.2	93.1	— .9	85.4	85.4
		1.06			2.06			1.89		
		1.44			1.23			1.51		

Fig. 1. Percentages of significant differences between the yields of crosses of sister progenies in successive generations of inbreeding.

TABLE 2. *Acre yield in bushels for the Krug top crosses of the selected and discarded sister sister progenies in the eighth selfed generation (indicated as No.*

Inbred parent	First			Second			Third		
	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.
L 287A1	81.6	......	....	58.5	65.5	— 7.0	58.6	69.2	—10.6
L 289A1	77.3	70.0	· 7.3	83.9	82.0	1.9	75.7	......	....
L 291	65.8	69.8	— 4.0	76.3	......	....	......	......	....
L 292A	84.5	69.8	14.7	76.1	58.9	17.2	72.9	68.7	4.2
L 293A1	66.2	54.7	11.5	65.1	47.7	17.4	71.7	......	....
L 295	55.7	79.4	—23.7	......	......	....	70.9	64.6	6.3
L 304A	81.7	78.1	3.6	82.4	81.2	1.2	93.0	91.2	1.8
L 309	69.1	53.1	16.0	57.1	75.7	—18.6	56.6	71.5	—14.9
L 311	64.1	......	....	58.8	74.8	—16.0	65.9	......	....
L 317B2	81.4	87.0	— 5.6	68.7	66.9	1.8	86.1	65.4	20.7
L 320A2	......	......	....	80.1	78.7	1.4	......	......	....
L 324	77.6	37.5	40.1	......	......	....	78.9	......	....
L 337	67.2	55.3	11.9	......	......	....	55.1	......	....
Mean differences			7.18			—.08			1.25
σ (Mean Diff.)			5.28			4.18			5.21

table 2 of 11.9 bushels may be considered significant. Frequency distributions of the numbers of significant differences in acre yield between the crosses of sister progenies in each generation are recorded in table 3 and shown graphically in figure 1. With continued inbreeding the yields of the crosses of sister progenies become more nearly equal. A rather large percentage of the differences are significant, even in the later generations of inbreeding. A portion of these significant differences without doubt are due to the inadequate sampling of the Krug variety in the individual crosses. There is no means of estimating, however, what portion of them are due to this factor.

TABLE 3. *Numbers and percentages of significant differences between the yields of crosses of sister progenies in successive generations of inbreeding*

Parentage of inbred lines	Generation of inbreeding					
	1	2	3	4	5	6
Iodent	7	2	3	1	2	0
Lancaster Surecrop	4	4	2	2	2	2
Totals	11	6	5	3	4	2
Percentage of differences significant	50	33	30	14	27	14

EFFECT OF SELECTION IN ISOLATING LINES MORE PRODUCTIVE THAN THEIR PARENTS

The effect of selection in isolating lines whose crosses were more productive than those of their parents was studied in the crosses of the selected progenies made after each generation of inbreeding. The data were complete for crosses of the selected progenies through all generations of inbreeding on 7 Iodent and 5 Lancaster Surecrop inbred lines. The data are presented in tables 4 and 5. Where data were available on

progenies from the first six selfed generations of the Lancaster Surecrop lines and of two 1 and No. 2) between which no selection had as yet been made

Fourth			Fifth			Sixth			Eighth	
Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	Sel.	Disc.	Dif.	No. 1	No. 2
73.8	56.1	17.7	64.6	57.8	6.8	79.7	85.0	— 5.3	55.6	95.6
83.8	79.1	4.7	77.9	87.6	— 9.7	......	......	....	71.3	......
66.3	76.7	—10.4	74.3	61.5	12.8	79.2	61.2	18.0	......	......
....	....	....	76.7	75.9	.8	79.5	......	....	68.8	......
79.9	69.2	10.7	66.6	......	....	72.1	83.7	—11.6	75.3	......
74.7	72.4	2.3	......	......	....	58.3	......	....	63.1	71.9
96.4	75.7	20.7	......	......	....	......	......	....	......	61.1
60.9	66.2	— 5.3	66.3	62.5	3.8	67.3	48.6	18.7	65.3	59.4
74.3	66.4	7.9	58.6	......	....	77.5	......	....	65.8	......
78.8	88.7	— 9.9	73.0	79.2	— 6.2	82.3	......	....	56.9	......
....	....	....	......	......	....	......	......	....	......	......
63.9	55.7	8.2	74.4	61.3	13.1	72.6	65.7	6.9	79.6	75.5
73.4	82.4	— 9.0	......	......	....	......	......	....	75.1	......
		3.42			3.06			5.34		
		3.30			3.32			6.09		

TABLE 4. *Summary of the acre yields in crosses after successive generations of inbreeding, Iodent lines*

Inbred parent	Acre yield (bushels) in the generation of inbreeding indicated							Means
	1	2	3	4	5	6	8	
I 154A	84.5	80.3	90.0	94.9	85.8	81.2	81.9	85.5
I 182	76.7	85.6	85.9	98.8	96.9	94.9	100.5	91.3
I 185B	63.4	80.1	81.3	72.7	70.2	79.5	77.7	75.0
I 197A1	76.6	91.0	84.0	68.9	92.5	79.1	75.5	81.1
I 234	74.2	87.3	78.4	88.9	91.9	89.6	89.2	85.6
I 238	73.4	86.4	81.4	80.3	87.8	89.1	81.5	82.8
I 252	95.6	88.8	96.9	90.6	73.6	92.2	85.4	89.0
Means	77.8	85.6	85.4	85.0	85.5	86.5	84.5	

TABLE 5. *Summary of the acre yields of crosses after successive generations of inbreeding, Lancaster Surecrop lines*

Inbred parent	Acre yield (bushels) in the generation of inbreeding indicated							Means
	1	2	3	4	5	6	8	
L 287A1	81.6	58.5	58.6	73.8	64.6	79.7	75.6	70.3
L 293A1	66.2	65.1	71.7	79.9	66.6	72.1	75.3	71.0
L 309	69.1	57.1	56.6	60.9	66.3	67.3	62.4	62.8
L 311	64.1	58.8	65.9	74.3	58.6	77.5	65.8	66.4
L 317B2	81.4	68.7	86.1	78.8	73.0	82.3	56.9	75.3
Means	72.5	61.6	67.8	73.5	65.8	75.8	67.2	

the crosses of 2 progenies in the eighth generation of inbreeding, the mean of the two values was used.

The analyses of variance for the data in tables 4 and 5 are shown in tables 6 and 7. The means of the yields of crosses for the Iodent lines (table 4) are about equal for the different generations of inbreeding except for the first generation, which is somewhat lower than the rest.

TABLE 6. *Analysis of variance of the acre yields of crosses with Iodent lines*

Source of Variation	D/F	Sum of squares	Mean square	Ratio to error
Total	48	3352.9012	69.8521	
Lines	6	1218.6983	203.1164	4.14*
Generations	6	368.3955	61.3993	1.25
Error	36	1765.8074	49.0502	

* Very significant.

TABLE 7. *Analysis of variance of the acre yields of crosses with Lancaster Surecrop lines*

Source of variation	D/F	Sum of squares	Mean square	Ratio to error
Total	34	2433.4617	71.5724	
Lines	4	632.3445	158.0861	3.57*
Generations	6	737.4057	122.9010	2.77*
Error	24	1063.7115	44.3213	

* Very significant.

The variance among the means for generations is not significantly larger than that due to error (table 6). The means of crosses for the different generations of the Lancaster Surecrop lines differ significantly, according to the analysis of variance in table 7, but show no particular trend. The means for both the Iodent and Lancaster Surecrop lines are shown graphically in figure 2.

Only those lines for which the data were complete were included in tables 4 and 5. Fragmentary data were available on additional lines which could be utilized by a different method of analysis. In table 8 are recorded the differences between the yields of the crosses of the selected progenies in successive generations for all of the Iodent and Lancaster Surecrop lines. Table 8 was made up from tables 1 and 2 and includes all of the lines where two successive generations could be paired. In each case the difference was determined by subtracting the earlier generation from the later; a positive difference therefore indicates that the cross of the later generation was more productive. Where data were available on two crosses of a line in the eighth generation, their

mean value was paired with the yield of the selected line after the sixth generation. The only significant mean difference occurs between the fifth and sixth generations and doubtless is due to chance. Four of the mean differences are positive and two are negative.
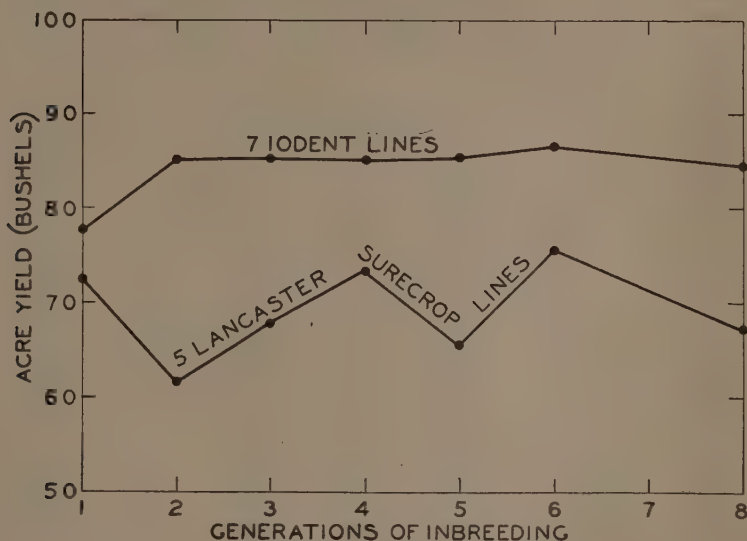
Fig. 2. The mean acre yields of the Krug top crosses of the successive inbred generations of 7 Iodent and 5 Lancaster Surecrop lines.

INDIVIDUALITY OF THE PARENT INBRED LINES

The productiveness of the different lines in top crosses differs markedly as shown in tables 4 and 5. The variances ascribed to lines (tables 6 and 7) are significantly greater than those due to error in both the Iodent and the Lancaster Surecrop groups. The complete data on acre yields recorded in tables 1 and 2 are shown graphically in figures 3 and 4, respectively, those for the crosses of each parent line being plotted separately. The horizontal dashed lines in these figures enclose a band twice the standard error of a difference (10.7 bushels in figure 3 and 11.9 bushels in figure 4) on each side of the yield of the cross of the selected progeny made after the second generation of selfing. For parent lines where no cross after two generations of selfing was available, the crosses of the selected progenies after either three generations or one generation of selfing were used.

Ten of the Iodent lines shown in figure 3 have data on crosses after the second and the sixth and eighth generations of inbreeding and one additional line has data on crosses after the second and eighth generation of inbreeding. For nine of these eleven lines the crosses after the sixth and eighth generations of inbreeding do not differ significantly

TABLE 8. *Summary of the differences between the acre yields of the top crosses made after successive generations of inbreeding*

Parent line	2nd minus 1st	3rd minus 2nd	4th minus 3rd	5th minus 4th	6th minus 5th	8th minus 6th
I 154A	— 4.2	9.7	4.9	— 9.1	— 4.6	0.7
I 163A2	16.0	—15.8	......	......	......	......
I 173B	— 5.8	......	......	0.2	3.1	— 2.5
I 182	8.9	0.3	12.9	— 1.9	— 2.0	5.6
I 185B	16.7	1.2	— 8.6	— 2.5	9.3	— 1.8
I 188B	......	......	......	......	......	......
I 197A1	14.4	— 7.0	—15.1	23.6	—13.4	— 3.6
I 211A	......	......	......	......	......	......
I 219	4.7	10.6	6.5	......	......	5.3
I 224A1	6.9	— 5.3	9.3	2.0	......	......
I 234	13.1	— 8.9	10.5	3.0	— 2.3	— 0.4
I 238	13.0	— 5.0	— 1.1	7.5	1.3	— 7.6
I 242A2	......	1.6	1.5	— 4.6	5.3	— 0.1
I 252	— 6.8	8.1	— 6.3	—17.0	18.6	— 6.8
L 287A1	—23.1	0.1	15.2	— 9.2	15.1	— 4.1
L 289A1	6.6	— 8.2	8.1	— 5.9	......	......
L 291	10.5	......	......	8.0	4.9	......
L 292A	— 8.4	— 3.2	......	......	2.8	—10.7
L 293A1	— 1.1	6.6	8.2	—13.3	5.5	3.2
L 295	......	......	3.8	......	......	9.2
L 304A	0.7	10.6	3.4	......	......	......
L 309	—12.0	— 0.5	4.3	5.4	1.0	— 4.9
L 311	— 5.3	7.1	8.4	—15.7	18.9	—11.7
L 317B2	—12.7	17.4	— 7.3	— 5.8	9.3	—25.4
L 320A2	......	......	......	......	......	......
L 324	......	......	—15.0	10.5	— 1.8	5.0
L 337	......	......	18.3	......	......	......
Mean differences	+1.61	+1.02	+3.10	—1.38	+4.18	—2.81
σ mean diff.	2.50	1.93	2.10	2.41	2.03	1.90

from those of the selected progeny after two generations of inbreeding, indicating that the individuality of the lines was established rather early in the inbreeding process. This is the same tendency as shown in the analysis of variance in table 6. The variance ascribed to "error" in tables 6 and 7 is in reality the interaction of line with generation. The fact that the variance due to line is significantly greater than that due to error indicates that the lines have definite tendency to maintain their respective placings in successive generations.

The data for the Lancaster Surecrop lines shown in figure 4 are more fragmentary than those for the Iodent lines and show greater fluctuation from generation to generation. Six lines have data on crosses after the second, sixth and eighth generations of inbreeding. For five of these lines, one or more of the crosses after the sixth and eighth generations of selfing differ significantly from those of the selected progenies after two generations of selfing. The fluctuations appear to be random, however, with no particular trend and may be due in part to
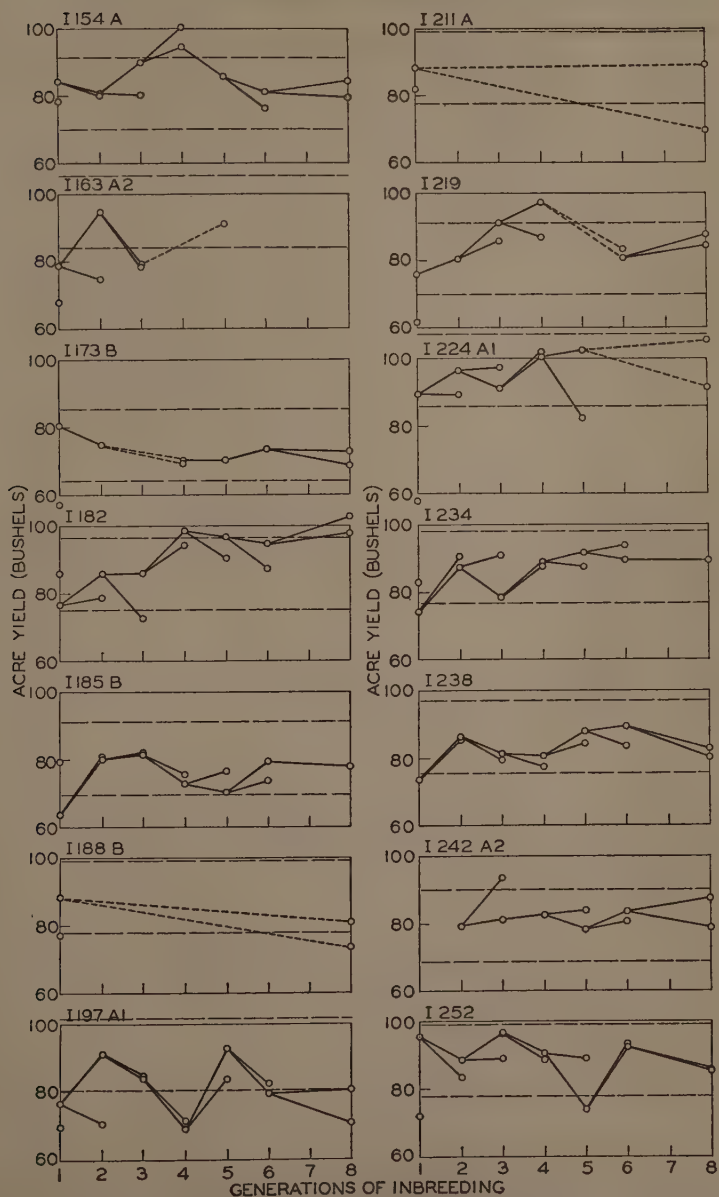
Fig. 3. Graphic presentation of the acre yields of the crosses of the Iodent lines after successive generations of inbreeding. Taken from the data in table 1.
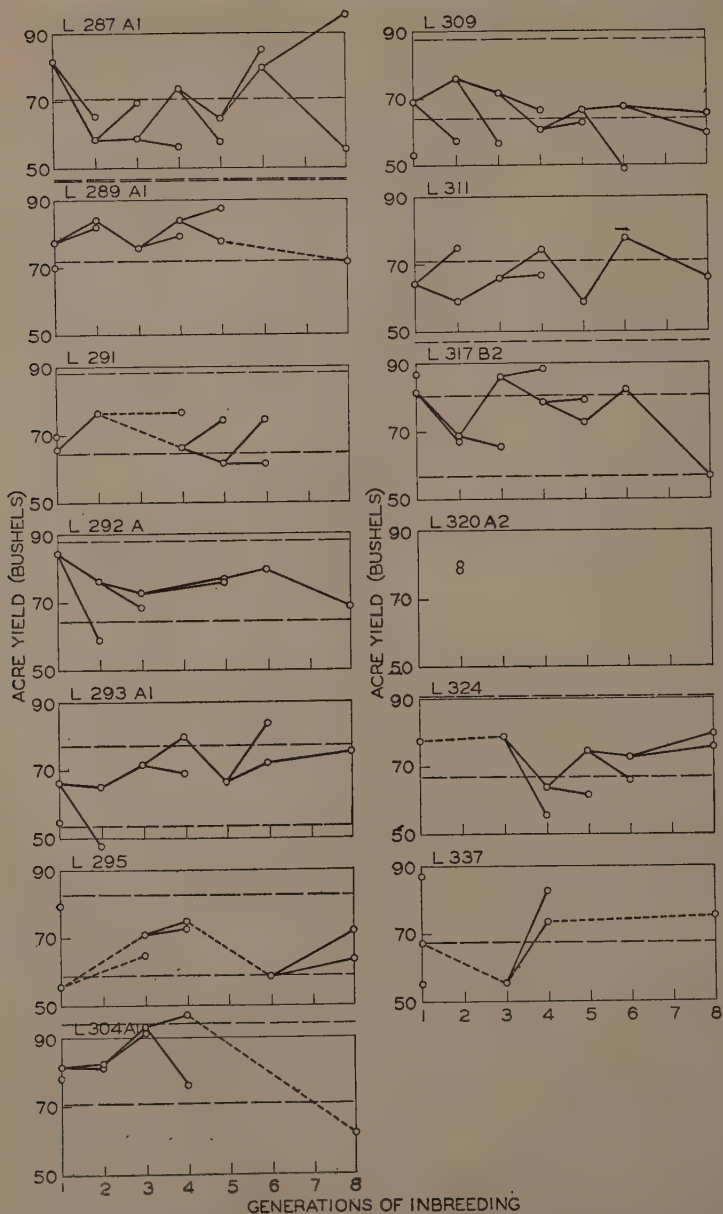
Fig. 4. Graphic presentation of the acre yields of the crosses of the Lancaster Sure-crop lines after successive generations of inbreeding. Taken from the data in table 2.

poor sampling of the Krug variety. The analysis of variance in table 7 here too, however, indicates a significant tendency of the lines to maintain their respective placings through the inbreeding period.

DATA ON CHARACTERS OTHER THAN YIELD

In addition to acre yield, data were taken on the following plant and ear characters of the crosses:

1. Plant height, inches
2. Nodes per plant, number
3. Lodging grade
4. Moisture in the harvested ears, percentage
5. Diplodia infected ears, percentage
6. Kernel rows per ear, number
7. Shelling percentage
8. Test weight per bu., pounds
9. Ears per cwt., number

The parent inbred lines had been subjected during their development to selection for resistance to lodging and freedom from moldy ears, whether due to diplodia or other ear rotting organisms. No particular selection had been practiced with regard to the other characters mentioned above so that the changes expected in these characters would be those incidental to the selection for yield, general plant vigor and resistance to lodging and disease.

Tables similar to 1 and 2 were prepared for each character and the mean differences between the crosses of sister progenies with their standard errors were determined. These are summarized in table 9. Differences two or more times their standard errors are printed in bold face type.

None of the differences for lodging grade or for diplodia infected ears, the two characters subjected to selection, are large enough to be considered significant. Of the 12 differences for lodging grade 9 are negative and indicate a tendency for the crosses of the selected progenies to be more erect (lower grades indicate less lodging) than those of the discarded ones. Of the 12 differences for percentage of diplodia-infected ears 11 are positive, indicating a consistent tendency for the crosses of the selected progenies to have more ear rot.

In the characters for which no particular selection had been practiced there were two significant differences following 1 generation of inbreeding, 2 following 2 generations of inbreeding and 3 following 5 generations of inbreeding. Inasmuch as the differences following the fifth generation of inbreeding in both the Iodent and Lancaster Surecrop lines occur in characters which in no case show significant differences in earlier generations, it would appear that they must be due to chance or to some extraneous cause.

Tables similar to 4 and 5 also were prepared for these characters. A summary of the means for each character after successive generations of inbreeding is recorded in table 10. Analyses of variance for the various characters similar to those for acre yield shown in tables 6 and 7 are summarized in table 11.

TABLE 9. *Summary of the mean differences between the top crosses of the selected and discarded sister progenies following the different generations of inbreeding*

Character	Mean differences following the generation indicated					
	1	2	3	4	5	6
Iodent Lines						
Plant height inches	-1.44 ± 1.47	1.92 ± 1.14	$-.24 \pm 1.35$	$.87 \pm 1.68$	-1.29 ± 1.77	$-.27 \pm 1.89$
Nodes per plant, number	$.07 \pm .15$	$.39 \pm .19$	$.03 \pm .24$	$.21 \pm .15$	$.06 \pm .16$	$.36 \pm .25$
Lodging grade	$.09 \pm .13$	$.19 \pm .13$	$-.05 \pm .13$	$.05 \pm .10$	$-.20 \pm .11$	$.08 \pm .09$
Moisture, per cent	$-.60 \pm 1.37$	$.84 \pm 1.06$	$-.86 \pm .78$	$-.63 \pm .56$	$1.19 \pm .54$	$.06 \pm .70$
Diplodia infected ears, p. ct.	$.09 \pm .66$	$.03 \pm .41$	$.12 \pm .74$	$.00 \pm .43$	$.08 \pm .57$	$.44 \pm .65$
Kernel rows per ear, number	$.55 \pm .42$	$-.66 \pm .46$	$-.10 \pm .50$	$-.04 \pm .21$	$.33 \pm .26$	$.33 \pm .24$
Shelling percentage	$.78 \pm .57$	$-.40 \pm .58$	$.10 \pm .40$	$.18 \pm .19$	$.14 \pm .24$	$.30 \pm .29$
Test weight per bu., pounds	$.53 \pm .25$	$-.10 \pm .29$	$.15 \pm .43$	$-.35 \pm .25$	$-.11 \pm .15$	$-.11 \pm .22$
Ears per cwt., number	-16.67 ± 8.34	-6.67 ± 9.94	-1.45 ± 5.47	3.90 ± 4.72	$.13 \pm 7.28$	-6.89 ± 4.45
Lancaster Surecrop Lines						
Plant height, inches	-1.80 ± 3.30	-2.13 ± 3.03	$-.30 \pm 1.80$	4.23 ± 2.19	$-.63 \pm 2.22$	1.20 ± 3.99
Nodes per plant, number	$.08 \pm .25$	$-.03 \pm .21$	$.13 \pm .28$	$.11 \pm .23$	$.47 \pm .08$	$-.04 \pm .21$
Lodging grade	$.08 \pm .38$	$+.13 \pm .56$	$.17 \pm .72$	$.02 \pm .61$	$.00 \pm .14$	$-.02 \pm .64$
Moisture, per cent	$1.12 \pm .87$	$.11 \pm .67$	$.98 \pm .52$	$.08 \pm .79$	$-1.71 \pm .87$	$.30 \pm 1.10$
Diplodia infected ears, p. ct.	$.73 \pm .84$	$.29 \pm .67$	$.38 \pm .79$	$.25 \pm .74$	$.70 \pm 1.09$	$.68 \pm 1.43$
Kernel rows per ear, number	$.00 \pm .34$	$-.49 \pm .41$	$-.50 \pm .30$	$-.10 \pm .31$	$.34 \pm .33$	$.32 \pm .38$
Shelling percentage	$.26 \pm .70$	$.73 \pm .62$	$.08 \pm .90$	$.75 \pm .50$	$.96 \pm .17$	$-.26 \pm .53$
Test weight per bu., pounds	$-.18 \pm .47$	$.97 \pm .46$	$-.47 \pm .79$	$.52 \pm .69$	$1.34 \pm .69$	$-.36 \pm .59$
Ears per cwt., number	-12.30 ± 8.33	7.33 ± 8.84	-6.83 ± 12.69	5.09 ± 7.55	-7.57 ± 10.19	-7.20 ± 8.90

TABLE 10. *Summary of the mean values for the characters others than yield*

Characters	Mean values following the generation indicated
	1	**2**	**3**	**4**	**5**	**6**	**8**
Iodent Lines							
Plant height, inches	83.1	82.8	82.8	82.5	81.6	86.4	81.9
Nodes per plant, number	15.8	15.6	15.4	15.4	15.2	15.3	15.2
Lodging grade	3.2	3.0	3.0	3.1	3.1	3.2	3.2
Moisture, per cent	25.3	25.5	24.3	24.4	24.7	25.6	24.4
Diplodia infected ears, p. ct.	1.5	.3	1.8	.8	.3	1.2	1.0
Kernel rows per ear, number	16.5	17.2	16.4	16.9	16.7	16.4	16.7
Shelling percentage	85.9	85.5	85.4	85.9	86.1	86.0	85.7
Test weight per bu., pounds	59.3	59.4	59.1	58.4	59.4	59.4	59.1
Ears per cwt., number	199.3	195.6	200.6	194.6	193.7	194.7	196.0
Lancaster Surecrop Lines							
Plant height, inches	84.6	87.3	90.6	89.1	89.4	92.1	89.4
Nodes per plant, number	15.2	15.2	15.4	15.4	15.4	15.4	15.3
Lodging grade	3.0	3.0	3.1	2.9	2.9	2.9	2.8
Moisture, per cent	24.6	27.4	27.7	27.1	26.2	27.3	27.0
Diplodia infected ears, p. ct.	2.8	2.0	2.4	3.1	1.6	1.3	0.6
Kernel rows per ear, number	15.5	15.3	15.7	15.5	15.9	16.0	15.8
Shelling percentage	84.0	81.5	82.1	81.7	81.9	82.0	81.3
Test weight per bu., pounds	60.1	59.5	59.5	59.0	59.9	59.0	59.2
Ears per cwt., number	211.0	230.6	220.8	210.8	226.0	207.2	213.4

The ratios of the variance due to generations to that due to error indicate no significant differences among the means for successive generations in either the Iodent or the Lancaster Surecrop lines. The ratios of the variance due to lines to that due to error indicate that the means of the crosses for lines differed significantly among the Iodent lines as regards nodes per plant, percentage moisture, kernel rows per ear, shelling percentage and test weight per bushel. The means of the crosses for lines differed significantly among the Lancaster Surecrop lines for nodes per plant, kernel rows per ear, shelling percentage, test weight per bushel and ears per cwt.

DATA ON VARIABILITY

The statement often is made that the selection ordinarily practiced in inbreeding projects with corn has a tendency to perpetuate the more heterozygous individuals. If this is the case, the crosses of the selected progenies might be expected to be more variable than those of the discarded progenies. Data were taken on individual plants (in two or three replications as indicated) and the variance computed for the following characters:

Plant yield, grams—3 replications
Plant height, inches—2 replications
Nodes per plant, number—2 replications
Kernel rows per ear, number—3 replications

The variances were assembled in tables similar to 1 and 2 and the mean differences between the variances of the top crosses for the selected

TABLE 11. *Summary of the variance between lines and between generations for the characters other than yield*

Characters	Mean squares			Ratios	
	Lines	Generations	Error	Lines Error	Gen. Error
Iodent Lines					
Degrees of freedom	6	6	36		
Plant height	16.7031	17.3979	8.7651	1.91	1.98
Nodes per plant	1.7204	.3709	.1728	9.96	2.15
Lodging grade	.5854	.0502	.4925	1.19	.10
Moisture	12.7066	2.0404	2.5046	5.07	.81
Diplodia infected ears	1.7608	2.1937	1.8299	.96	1.20
Kernel rows per ear	6.1656	.5370	.5364	11.49	1.00
Shelling percentage	2.9797	.4340	.5442	5.48	.80
Test weight per bu.	7.7733	.9061	.5246	14.82	1.73
Ears per cwt.	281.4762	46.4762	190.2857	1.48	.24
Significant ratios*					
5 per cent point				2.36	2.36
1 per cent point				3.36	3.36
Lancaster Surecrop Lines					
Degrees of freedom	4	6	24		
Plant height	44.7453	28.4265	21.7008	2.06	1.31
Nodes per plant	2.2007	.0510	.1016	21.66	.50
Lodging grade	.3025	.0338	.1339	2.26	.25
Moisture	11.0761	5.6472	2.7150	4.08	2.08
Diplodia infected ears	2.7347	3.7423	4.3896	.62	.85
Kernel rows per ear	5.4833	.3272	.2788	19.67	1.17
Shelling percentage	16.4269	4.0080	2.7992	5.87	1.43
Test weight per bu.	7.0954	.9000	.9739	7.29	.92
Ears per cwt.	1,306.9572	386.4572	225.0405	5.81	1.72
Significant ratios*					
5 per cent point				2.78	2.51
1 per cent point				4.22	3.67

* From Tables by Snedecor, G. W. Analysis of Variance. Collegiate Press, Inc., Ames, Iowa. 1934.

and the discarded sister progenies were determined. These mean differences with their standard errors are recorded in table 12. Differences 2 or more times their standard errors are printed in bold faced type.

Only two of the mean differences are significant as judged by their standard error. Taken as a whole the differences indicate the relative variability of the crosses of the selected and the discarded progenies to be about equal. Either the selection practiced has not tended to perpetuate the more heterozygous individuals or the methods of measurement used have not been sufficiently precise to demonstrate such a tendency.

Tables similar to 4 and 5 were prepared and the means of the variances after successive generations of inbreeding were determined. These means are summarized in table 13. The data on the variability were subjected to analyses of variance, similar to those shown in tables 6 and 7. These analyses are summarized in table 14.

TABLE 12. *Summary of the mean differences between the variances of the top crosses of selected and discarded sister progenies in the different generations of inbreeding*

Characters	Mean differences following the generation indicated					
	1	2	3	4	5	6
Iodent Lines						
Plant yield, grams	−618.1 ±1181.7	−1752.1 ± 443.2	1507.5 ±1367.5	−427.8 ±903.1	−1070.5 ±2290.6	490.7 ± 951.8
Plant height, inches	4.41± 6.30	2.70± 3.42	6.48± 5.04	− 6.39± 5.22	6.93± 5.40	− 1.80± 7.29
Nodes per plant, number	.24± .16	.05± .12	− .07± .19	.01± .14	.07± .17	.12± .13
Kernel rows per ear, number	.46± .59	− .93± .69	− .12± .41	− .58± .47	.10± .60	− .75± .30
Lancaster Surecrop Lines						
Plant yield, grams	−997.8 ±1083.6	425.5 ±1348.5	1531.6 ±1483.8	303.4 ±472.8	−2367.9 ±4959.4	−609.9 ±1550.7
Plant height, inches	3.42± 7.83	− 8.19± 11.16	4.59± 13.50	− 1.44± 6.93	1.08± 13.23	− 5.40± 3.87
Nodes per plant, number	− .02± .18	− .04± .11	− .18± .29	.00± .11	.12± .13	.04± .11
Kernel rows per ear, number	.17± .50	− .60± .62	− .71± .63	− 1.05± .71	.37± .34	.32± .22

TABLE 13. *Summary of the mean variances for the characters studied*

Character	Mean variance following the generation indicated						
	1	2	3	4	5	6	8
Iodent Lines							
Plant yield, grams*	107.72	72.84	90.52	89.59	70.91	74.74	93.06
Plant height, inches	51.39	47.70	52.65	47.97	58.23	52.11	45.18
Nodes per plant, number	1.60	1.29	1.37	1.43	1.21	1.23	1.19
Kernel rows per ear, number	4.61	4.73	3.78	3.86	3.79	3.30	3.73
Lancaster Surecrop Lines							
Plant yield, grams*	113.10	119.07	113.35	110.18	106.22	96.72	121.92
Plant height, inches	62.64	72.54	54.00	59.31	58.68	60.12	43.20
Nodes per plant, number	1.38	1.34	1.38	1.08	1.33	1.35	1.05
Kernel rows, per ear, number	3.80	4.06	3.84	4.28	3.37	3.63	3.61

* The values in this line have been divided by 100.

TABLE 14. *Summary of the variance between lines and between generations for the variances of the four characters measured*

Characters	Mean squares			Lines Error	Gen. Error
	Lines	Generations	Error		
Iodent Lines					
Degrees of freedom	6	6	36		
Plant yield	10,510.8195*	12,618.9054*	5,389.7470*	1.95	2.34
Plant height	294.8400	127.1295	174.7980	1.69	.73
Nodes per plant	.0473	.1565	.0952	.50	1.64
Kernel rows per ear	1.0999	1.8353	1.1652	.94	1.58
Significant ratios**					
5 per cent point				2.36	2.36
1 per cent point				3.36	3.36
Lancaster Surecrop Lines					
Degrees of freedom	4	6	24		
Plant yield	5,385.9758*	3,500.0392*	4,667.0239*	1.15	.75
Plant height	864.9018	394.6401	352.5039	2.45	1.12
Nodes per plant	.0636	.1056	.1012	.63	1.04
Kernel rows per ear	4.1005	.4560	.6316	6.49	.72
Significant ratios**					
5 per cent point				2.78	2.51
1 per cent point				4.22	3.67

* Divided by 1,000.
** Interpolated from tables by Snedecor, G. W. Analysis of Variance. Collegiate Press, Inc., Ames, Iowa. 1934.

The ratios of the variances due to generations to those due to error indicate no differences in the variability of crosses made after successive generations of inbreeding. The use of open-pollinated Krug as one parent of the crosses, however, may have introduced so much variability as to mask any change in variability due to selection or to continued inbreeding.

DISCUSSION

The data presented represent an attempt to measure the effects which the inbreeding and selection practiced in the Iowa breeding program have had upon the crosses made after successive generations of inbreeding. The procedure of testing the lines in top crosses was used because of the simplicity of the method. The results probably have been affected by the inadequate sampling of the Krug variety in some cases and the performance of individual combinations must be interpreted with this in mind. It would seem, however, that the general averages for generations, lines, etc., should be reasonably reliable.

The more important conditions brought out by the data are:

1. Selection between sister progenies was effective in choosing the progenies whose crosses were slightly but consistently more productive than those of their discarded sibs. The crosses of the selected progenies also were more erect but were more susceptible to diplodia.

2. Selection was ineffective in improving strains so that their crosses differed consistently from those of their parent in productiveness or in any of the other characters studied. The inbred lines, particularly the Iodent lines, acquired their individuality as parents of top crosses very early in the inbreeding process and remained relatively stable thereafter. Fluctuations in the productivity of the crosses of the later generations appear to have been random, at least they show no particular trend.

The general ineffectiveness of the selection in influencing the top crosses of subsequent generations is a disappointment to the plant breeder. It might reasonably be assumed that with continued inbreeding *and selection* the yield of the hybrids of progenies from the later generations of inbreeding should have been larger than the yield of those from the earlier generations. There is no indication that this was the case. Apparently the effective selection between sister progenies has been successful only in preventing the yields of crosses from the later generations in actually being lower than those from the earlier generations. These results do not minimize the need for or the effects of the selection upon the parent lines themselves. They do emphasize the necessity, however, of basing selection for performance in hybrids upon the results of crossing trials rather than simply upon the characteristics of the lines themselves.

The early individuality of the lines should permit testing them in crosses earlier than usually has been the case, with the consequent earlier elimination of unpromising lines. Such a procedure should permit a concentration of effort on the lines more promising in hybrid combination.

The early stability of the lines was not expected. Two possible explanations suggest themselves. The first is based upon the assumption that the total complement of yield factors is rather small and that very early in the inbreeding process the lines became homozygous for certain

major factors and changed little thereafter. This explanation is not in keeping with present information regarding quantitative characters. "Student"[4] has recently estimated that from 100 to 300 genes control oil and protein content in maize on the basis of the Illinois selection experiments. Certainly yield and the other characters considered in these experiments should be controlled by no fewer genes. Data already presented showed that there were significant differences between the crosses of sister progenies for too many generations to assume that the parent lines were homozygous.

A second and what seems to the writer a more reasonable explanation is based on the assumption that yield (and the other quantitative characters studied) is controlled by a larger number of factors, many of them having approximately equal influence. As selfing drove these into fixation, the selection practiced merely sampled the modal classes in each generation.

The various plants in an open-pollinated variety of corn (or the segregating generations following a cross) will have different complements of genes affecting the different characters. The inbreeding process theoretically should drive equal numbers of dominants and recessives into fixation. The fixing of recessives results in reduced vigor of the inbred lines. The operation of chance in the selection of the particular dominant genes to be fixed among a group of dominants of approximately equal value, however, should not change the performance of the line in crosses. For each dominant gene lost through being fixed in the recessive condition another theoretically will be fixed in the homozygous dominant condition and thus the number of dominant allelomorphs present will remain unchanged.

As an example, assume that genes A, B, C and D have essentially equal influence upon a quantitative character such as yield. If a strain of corn heterozygous for these 4 factors is selfed, the resulting inbred lines will, on the average, have 2 of them fixed in the homozygous dominant condition and 2 in the homozygous recessive condition. The average of a large number of crosses (or the top cross) of a line of the composition $AA\ BB\ cc\ dd$ should be equal to that of a line of the composition $aa\ bb\ CC\ DD$ or for that matter the average for either of these lines should be equal to that of the parent from which they were developed, which was $Aa\ Bb\ Cc\ Dd$. In each case 4 dominant genes of equal influence are brought into the crosses and the end results should be equal. The relative vigor of the parent lines might be very different, however. The crossing results should be similar regardless of whether it be assumed that the effect of the different factors is additive or is cumulative in some such manner as suggested by the interaction hypothesis of Rasmusson[5]. The question of "nicking" is not considered, as the discussion is concerned with average performance in combination with a broad range of germ plasm. Under these conditions "nicking" is of little importance.

This explanation of the individuality of the lines in crosses with regard to quantitative characters is based upon the added assumption of

[4] "Student." Evolution by Selection. Eugenics Review 24: 293-296. 1933.

[5] Rasmusson, J. A contribution to the theory of quantitative character inheritance. Hereditas 18: 245-261. 1933.

maintaining a rather fixed *number* of dominant genes throughout the selfing process instead of confining it entirely to the perpetuation of *particular* dominant genes. If it is correct, the differences between lines will in a large measure be those which exist in the plants from which the lines are started. The early individuality of the lines should make possible the earlier testing of them and the elimination of undesirable lines before much effort has been spent upon them.

The data indicate greater possibilities for progress in selection *among* lines rather than *within* them. Any modification in practice which would facilitate selection among larger numbers of lines or a greater concentration of effort on the most promising lines should aid progress in the development of better lines. The selection in the early generations might be concentrated on selection among lines based on crossing tests and that in the later generations might have as its primary object the improvement of those characters which "dress up" the line and its crosses.

The newer lines begun at the Iowa Station are now being tested in top crosses after two generations of selfing. The top crosses might be made after one generation of inbreeding, however, or might even be made at the time the open-pollinated plants are inbred the first time. Under such a procedure the open-pollinated plants might be both selfed and used as the pollen parents of top crosses. The similarity between such a procedure and the ear-to-row tests of previous years is at once apparent. Ear-to-row tests determined the yields of crosses between individual plants and the parent variety, the individual plants being used as seed parents. In the ear-to-row procedure, however, the identity of the individual plants as sources of breeding lines had been lost when the test was completed. In the procedure suggested above their identity would be preserved. The numerous published results of ear-to-row tests have offered ample promise for corn improvement if the identity of the better lines were to be maintained.

SUMMARY

Two progenies from the first to eighth generations of inbreeding, inclusive, except for the seventh, of 14 inbred lines each of Lancaster Surecrop and of Iodent were top crossed with Krug. One of these progenies from each inbred line in each generation was the selected progeny representing the direct line of descent. The other represented a sister progeny chosen at random from among those discarded in favor of the one selected to continue the pedigree.

Data were taken on a number of characters in the top crosses in order to study (1) the effect of inbreeding and (2) the effect of selection within the parental lines upon hybrids involving them.

Selection between sister progenies was effective in isolating those progenies whose crosses were slightly but consistently more productive than those of their discarded sibs.

Selection was ineffective in isolating strains whose crosses differed from those of their parents in productivenss or in any of the other characters studied.

The inbred lines acquired their individuality as parents of top crosses very early in the inbreeding process and remained relatively stable thereafter.

The data indicate that selection for performance should be based upon crossing tests rather than upon the appearance of the parent lines.

The early individuality of the lines in crosses should permit their early testing, possibly after the first and certainly after the second generation of inbreeding.

This early stability of the lines in crosses is explained on the basis of the *numbers* of dominant genes present as well as the *particular genes* present. Essentially equal numbers of dominant allelomorphs will be preserved through the successive generations of selfing.

SOME NEW MUTANTS IN MAIZE[1]

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Mutations as a source of new variations for organic evolution are gradually assuming more importance, particularly in the newer statistical philosophy of genetics. In the early days of the mutation hypothesis, it scarcely seemed reasonable that the few, crude, retrogressive mutants could be of any positive value. With the discoveries that genes have manifold effects, and that the drift of the genes through an amazing number of gene complexes provides infinite possibilities of adaptation in such a trial and error selective process, it became evident that new mutant genes, even though they exhibit a superficial, retrogressive change, may nevertheless serve as good building stones for entirely new combinations of adaptive gene changes. We still need, for such generalizations, more material and more data covering a wider range of mutant changes. Especially do we need further evidence on the dominance relations of new mutants.

During the past two decades, research with inbred lines of maize has provided a fertile source for the isolation of new mutants under controlled conditions. In the present report, descriptions and genetic experiments with eight new mutants, four dominants and four recessives, are presented with the sole object of recording and adding to our knowledge of hereditary mutations. These mutants involve variations in chlorophyll, morphology, anthocyanin, sex and carbohydrates.

DOMINANT MUTATIONS

1. *Dominant chlorophyll 'old-gold' striping.* In 1932, a single mutant plant occurred in a standard, inbred yellow-dent line (Idt) which had been selfed for the preceding 9 generations and sib-pollinated the 10th generation. The first 5-6 leaves were full green and then a light green or yellow striping began to emerge in the younger leaves. This striping became increasingly broader and yellower in the succeeding leaves until the last two leaves just below the tassel were practically pure golden-yellow. The plant produced abundant pollen.

When outcrossed with another standard, inbred line (Ldg), the offspring showed a clean-cut 1:1 ratio of yellow-striped (old-gold) and full green plants (total 56 old-gold striped : 47 green). This F_1 generation exhibited the expected heterosis and uniformity of a single cross, with the exception that the striped plants were slightly reduced in size and vigor compared with the greens.

Evidently the original mutant plant was heterozygous, the new striping being dominant. Here we have a case of the rare mutation from recessive to dominant. Only one other dominant chlorophyll mutant has

been reported (Kempton 1924) and that was a lethal yellow seedling, the original plant having been a sectorial chimera.

From the 1:1 progeny of the original cross (Ldg inbred x mutant Idt), 4 striped plants and 3 greens were self-pollinated. The former gave a progeny consisting of 322 striped, old-gold, and 106 greens. The latter bred true green. The new mutatnt may accordingly be described as a monogenic dominant (gene symbol *Og*). Apparently the homozygous dominant is not lethal since it is as equally capable of growth as is the heterozygote, both being fully green in the seedling stage.

2. *Dominant Teopod.* Two new sources of the already reported (Lindstrom 1925) Teopod mutant are now available. The original mutation was discovered in Wisconsin in 1921 where a farmer had isolated a single Teopod plant and grown two generations of it, before it came into the possession of the writer. This proved to be a monogenic, dominant variation, giving fairly clean-cut segregation.

The second discovery of a Teopod type came from a single, freak ear in the 1930 Iowa Corn Show (Ames), evidently having arisen in a yellow dent variety. A third specimen appeared in the 1931 Corn Show, also of yellow dent type, but from a different Iowa county. There is no assurance that the second and third sources were independent beyond the facts that the exhibitors were farmers from different counties, and no interchange of seed had ever been made between the two farms, as well as that the two ears were different in appearance.

The second specimen had the characteristic Teopod glumes or bracts over the entire ear, whereas, the third was bracted only on the lower third of the ear.

These open-pollinated ears were planted and both gave progeny with slightly less than 50 per cent Teopod-like plants. In general the staminate and pistillate inflorescences were characteristically like Teopod, but the leaves were broader and the tillering less than that of the original Teopod. The plants were described as coarse-leaved Teopod (Pl. I).

The segregation was not as clean-cut as that of the original Teopod material. Controlled crosses with normals gave an approach to a 1:1 ratio but always with an excess of normal plants. Self-pollinated Teopods gave also an excess of normals. Evidently the gene complex of modifying factors was not as well balanced as was the case in the original Teopod.

Crosses of the new Teopod-like variants with the original stock of Teopod, indicate that the same basic dominant *Tp* gene is involved. But the progenies of these crosses exhibited the variations noted above and classification was not always certain. There is, however, no doubt as to the major, single, dominant gene basis for all the Teopods, since all the progenies from backcrosses to normals gave the same ratios.

As has been noted in the original report, the importance of this dominant mutant lies in the tremendous influence of a single gene. Here is a gene that produces a morphological effect worthy of species differentiation on a phenotypic basis. That this same gene should have mutated a second and possibly a third time is an interesting phenomenon.

Data on the linkage relations of the *Tp tp* pair of genes are now available. The gene is on the 7th chromosome, a three point test of three linked genes giving the following order and distances, *Ra*—8.8—

Tp—5.7—*Ij*. There is 13.2 per cent crossing over between the extreme genes *Ra* and *Ij* (table 1).

3. *Dominant sorghum tassel* (Pl. II, fig. 1). Another freak ear in the 1931 Corn Show had a sorghum-like tassel, with numerous kernels about the size of ordinary BB shots, a trifle smaller than average sorghum seeds. When planted, this open-pollinated seed gave rise to slightly less than 50 per cent sorghum tassel plants. Six of these were crossed with normal, inbred lines of various genetic composition. In every case the sorghum-like plants were heterozygous and these progenies gave a total of 61 sorghum-like tassels and 43 normals, demonstrating that this form was also a monogenic dominant, the first described, although Emerson has mentioned two dominant tassel-seed forms.

This dominant mutant resembles Hayes' recessive sorghum-tassel type except that the dominant is more productive of seeds in the tassel. In both, the usual pistillate inflorescense is functional but the staminate parts in the tassel are suppressed whereas silks with ovaries are plentiful on the sorghum-like tassel. The regular pistillate ears develop better when this seed-bearing tassel is removed.

TABLE 1. *Dihybrid summary of three-point backcross linkage test of Teopod, Iojap and Ramosa genes on the 7th chromosome; coupling phase*

| Genes | | XY | Xy | xY | xy | Total | No. recomb. | Percentage |
X	Y							
Tp	Ij	163	9	9	137	318	18	5.7
Tp	Ra	171	1	27	119	318	28	8.8
Ra	Ij	164	34	8	112	318	42	13.2

This new, dominant gene is essentially another 'sex' gene, being one of 6-7 others that control the expression of the sporophytic 'sex' characters in maize (Emerson 1920, 1924, Phipps 1928).

While its linkage relations have not been determined so that one could positively state that it was genetically different from other dominant tassel-seed or tassel-ear types, its phenotypic differences are sufficiently different to suggest that it is a new dominant variation. It does not particularly resemble the recessive tassel-seed or tassel-ear forms described by Emerson (1920).

4. *Dominant pericarp mutation* (Pl. II, fig. 2). This is a striking bud mutation arising in one ear of an open-pollinated yellow-dent (non-variegated) type. Similar mutations in calico or variegated corn are very common, but this change from recessive P^{wr} (colorless pericarp, red cob) to the dominant P^{rr} (red pericarp, red cob) gene is rare. Brink (1929) has reported a similar mutation but in the opposite direction, from dominant to recessive.

When seeds from the red mutated area were planted, 7 full red ears and 7 colorless pericarp-red cob types emerged. From the colorless pericarp (yellow kernels) 11 similar colorless-pericarp plants resulted, prov-

ing that it was the unusually stable recessive P^{wr} gene which had mutated to the completely dominant P^{rr} gene.

RECESSIVE MUTATIONS

5. *Recessive anthocyanin* (a_3). The known, basic anthocyanin (or fla-vone) plant colors in maize are dominant to the non-colored, or paler shade of red (or purple). Anthocyanin genes A_1, A_2, B and Pl all show dominance to their allelomorphs. This new recessive mutant emerged in the inbreeding process with a line of sweet corn, characterized by the pseudo-starchy type of kernel.

In appearance it resembles the full sun-red of Emerson $(A \ B \ pl)$, but it is completely hypostatic even to the dilute sun-red $(A_1 \ b \ pl)$. When crossed with the brown type $(a_1 \ B \ Pl)$ it gives 100 per cent full purple, showing that it carries the basic A_1 gene. Its interrelations with the A_1 $B \ Pl$ genes are not yet fully worked out. That it is different from each is proved by the fact that it is borne on a totally different chromosome from each of the others. Gene a_3 is on the 10th chromosome, lying beyond R and G.

The data on the new recessive red are included in table 2, where there are also some new data on certain other genes on the 10th chromosome. The order of these genes is L_2—26—W_2—16—R—11—G—23—A_3. Genes li (lineate) and A_3 have not been tested, but should be closely linked.

6. *Recessive albino seedling* (w_4). This is a new pure albino gene that was isolated by inbreeding in a yellow dent variety. Being linked with the sugary gene and being the only albino gene on the 4th chromosome, it has been designated w_4. It shows 37.0 ± 0.9 per cent crossing over with su (table 3).

7. *Recessive "albescent" chlorophyll* (al). This new gene was isolated from an Illinois variety (Carter's strain) of yellow dent corn in the in-breeding process. It is fully green in the seedling stage and after the 5th-7th leaf, begins to show a fine streaking of white. The older leaves become increasingly whiter (but not pure white), giving a silvery-green appearance. It produces abundant pollen, and under good growing con-ditions a small ear.

Albescent is probably in the Y linkage group, a limited amount of data showing 42.3 ± 3.6 per cent crossing-over with this yellow endo-sperm gene. While it may resemble slightly the "fine-streaked" recessive type also found on this same chromosome, it is different enough pheno-typically, and its looser linkage with Y indicates that it is a different gene. It has not been crossed with fine-streaked, however, nor with an albescent type noted by Phipps but not described by him.

8. *Recessive sugary* (su). In 1928 occurred a single kernel mutation from dent to sweet corn under controlled pedigree conditions. Thirty yellow-dent inbreds were pollinated by inbred Evergreen sweet corn during that year. Among these 30 ears, comprising about 18,000 kernels, a single sweet corn kernel was found. Since the maternal parents of these crosses were all pure dent corn inbreds, this single sweet kernel obviously was not due to contamination, but must have arisen as a mutation in the megaspore or megaspore mother cell. The mutation struck only one kernel, involv-ing both the egg and the primary endosperm nucleus.

TABLE 2. *Summary of 10th chromosome linkages*

Pedigree	Genes X	Genes Y	Link. phase[1]	XY	Xy	xY	xy	Total	Recombination No.	Recombination Pctg.
6178	W₃	L₂	S R	935	420	......	......	1355		26.5
7781	W₂	L₂	S R	759	342	......	......	1101		26.1
7690	W₂	G	S R	1340	623	......	......	1963		21.8
6610	R	W₂	S C	435	45	46	103	1429		16.0
7690	R	W₂	S C	1660	152	182	334	2328		16.8
6613	R	L₂	S R	1254	553	596	75	2378		32.8
7905	R	L₂	S C	1277	270	323	247	2117		32.9
7882	L₂	G	S R	584	212	......	......	796		44.8
7905	L₂	G	S R	1323	461	......	......	1784		47.0
7884	R	G	S R	504	277	277	3	1061		11.0
9415	G	A₃	S R	260	65	96	3	424		22.5
9451	R	A₃	S C	120	24	49	20	213		40.3
8274	G	Li	B C	222	45	50	211	528	95	18.0
6426	G	Li	B R	57	253	208	23	541	80	14.8

[1] C = coupling, R = repulsion, S = selfed F₁, B = backcross.

TABLE 3. *Linkage of Su su and W₄ w₄ genes*

Pedigree	Su W₄	Su w₄	su W₄	su w₄	Percentage crossing–over
9232	420	197	188	31	35.5
9510–3	675	278	284	50	38.1
9597–9	505	185	184	25	35.1
9699	766	317	324	53	37.0
Total	2366	977	980	159	37.0 ± 0.9

This one seed was carefully planted in the greenhouse in 1929, and the resulting plant showed the characteristic hybrid vigor of a single cross. It grew to be over 8 feet tall. The summer of 1929 was abnormally hot, and the single tassel growing to touch the glass roof of the greenhouse fired completely.

A pollen mixture of Evergreen and Black Mexican sweet corn pollen was applied. The resulting ear had approximately 300 kernels, all sweet corn. Since that time, four generations of self-pollination have yielded nothing but sweet corn, and inbred lines of white or purple sweet corn, with red and with white cobs are available.

Here, then, is another case of mutation from a dominant *Su* to a recessive *su* gene, occurring with a frequency of 1 in 18,000, a sample that is too small to afford reliability on the mutation rate. The mere fact of its occurrence, however, is interesting in that one need not assume that our present sweet corns had a prehistoric origin. The absence of true sweet corn in archeological exhibits also would seem to indicate a relatively recent origin of sweet corn, with which the mutation process is in full agreement.

SUMMARY

Four new dominant, monogenic mutations are described. One is the first, non-lethal, dominant chlorophyll ("old-gold" striping) mutant to be reported. The second is the recurrence of the striking Teopod mutant, reported for the first time 12 years ago. A third dominant is a "sex" gene, sorghum-tassel, giving essentially a wholly pistillate form of maize. The fourth is a pericarp color mutation from the recessive P^{wr} to the dominant P^{rr} gene.

Of the four dominant mutations, only two (the chlorophyll and the pericarp forms) are fertile in the homozygous dominant condition.

The four new recessive mutations are all monogenic. One is a new, recessive anthocyanin variation, similar to the dominant, well-known sun-red. It is located on the 10th chromosome. A second recessive is a new, lethal albino seedling linked with sugary on the 4th chromosome. The third is a new, white-streaked (albescent) chlorophyll type linked with the Y endosperm color gene. The fourth mutant is a recurrence of the sugary gene under controlled conditions, having arisen in a long-inbred strain of yellow dent corn in one of 18,000 female gametes (megaspores).

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PLATE I

Teopod maize plants. Fine-leaved type on the left; coarse-leaved type on the right.

PLATE I

PLATE II

Fig. 1. Dominant sorghum-tassel mutant. Pistillate inflorescence on left; tassel with functional kernels on right.

Fig. 2. Pericarp bud mutation. Original mother ear was genetically colorless pericarp-red cob. Ear on left from yellow sector; ear on right from red sector.

PLATE II

DISSEMINATION OF BACTERIAL WILT OF CORN

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One of the most striking plant-disease developments of the past three or four years was the unprecedented distribution and destructiveness of bacterial wilt of corn during 1932 and 1933 [1-7], (18, 19, 23, 32, 33, 34). This was the climax of more than 35 years of intermittent outbreaks. During that time the disease has been studied by a number of able investigators, and it seems worth while at this time to review some of their work and trace briefly what we know regarding the history and development of the disease.

Bacterial wilt has developed chiefly as a disease of sweet corn. During the period from 1880 to 1900 the sweet-corn industry was developing rapidly in the Central and Eastern States, the first plant pathologists were beginning their work at Federal and State experiment stations (40), and the study of bacterial diseases of plants was just beginning (4). In 1897 (37) the first epidemic of bacterial wilt of sweet corn was reported. Stewart found the disease widespread and abundant on Long Island from 1895-97 and concluded that it probably was widely distributed.

Field corn is one of the oldest food plants and was extensively cultivated in North and South America in pre-Columbian times (38). Sweet corn, on the other hand, appears to be a recent development (10, 11, 38). Only one specimen of sweet corn has so far been identified from the numerous archeological collections of maize recovered in the United States (11).

Only four varieties are reported to have been grown by the Indians of the upper Missouri (41) and two by the Iroquois (22). They were apparently not used as green corn and their cultivation probably was limited. "A study of the literature covering the food crops of the American Indians indicates the limited number of forms [of sweet corn] which could have reached the hands of our early farmers. If the white-

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[1] 1932. Bacterial wilt of sweet corn *(Aplanobacter stewarti)*. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 16: 140-142.

[2] 1932. Bacterial wilt of sweet corn *(Aplanobacter stewarti)*. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 16: 167-168.

[3] 1933. Bacterial wilt of corn. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 17: 97-98.

[4] 1933. Bacterial wilt of corn reported from Maine. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 17: 109.

[5] 1933. Bacterial wilt of corn. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 17: 138-140.

[6] 1933. Further reports on bacterial wilt of corn. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 17: 151-155.

[7] 1933. Bacterial wilt of corn in Michigan and Massachusetts. U. S. Department of Agriculture, Bureau of Plant Industry, Plant Disease Reporter 17: 164-165.

kerneled, red-cobbed form carried from the fields of the Susquehanna to Plymouth in 1779 was the first sweet corn known to the settlers, then all named varieties have originated within the last 150 years" (38). Eight-ten rowed types with black, white and brownish red kernels and 10-16 rowed sorts with yellow and cream kernels came to the early settlers from the Indians, but "the total number of named varieties known during the fore part of the [past] century possibly did not exceed 10" (38). One variety was listed for sale for the first time in Thorburn's Catalog in 1828. Seven named varieties including Stowell's Evergreen were added up to the time of the Civil War. In 1884, Sturtevant published a list of 33 varieties. From that time on the number increased rapidly until at the present time over a thousand varieties have been named (38).

In 1884 and 1885, Sturtevant (1, 2) was testing sweet corn varieties in New York, and from 1888 to 1890 Burrill and McCluer (6) carried on variety tests in Illinois. Sweet corn canning (20) began in Maine in 1845, at Elgin, Ill., in 1861 and at Vinton, Iowa, in 1879. Up to 1900 sweet-corn varieties were almost entirely of the white-kernel type. Late varieties such as Stowell's Evergreen are resistant to bacterial wilt and possess endosperm characters which indicate that they have descended from the resistant dent corns. With the introduction of Golden Bantam in 1902 (38), yellow varieties especially susceptible to bacterial wilt became more popular. Some of these early susceptible varieties apparently have been developed from flints, which also are very susceptible to wilt. The increasing intensive cultivation of early susceptible varieties of sweet corn around the larger centers of population and in canning areas throughout the Central States greatly increased the opportunities for the spread of bacterial wilt.

Early reports of injury to field corn in Illinois are very suggestive of bacterial wilt although the disease was not reported from that state until a number of years later. Burrill in 1889 (5) described a number of symptoms characteristic of bacterial wilt but if he did observe this disease he confused it with other corn injuries and described an organism different from *Aplanobacter stewarti*. The reports of the State Entomologist of Illinois for 1892 (12) and 1905 (13) on Noxious and Beneficial Insects of Corn, state that the flea beetle, *Chaetocnema pulicaria*, was particularly abundant in central and southern Illinois in May and June of 1891. The beetles attacked young corn plants 5 or 6 inches high, killing the terminal part of the leaf beyond the feeding injuries. "Whole fields were *wilted* more or less and some hills entirely killed." McCluer in 1892 (21) reported that Burrill's bacterial disease of corn occurred in his sweet corn plots at the University of Illinois in 1891. "Plots 4, 5 [Cory], 7, and 8 [Burlington] were very badly affected by the bacterial corn disease, described in bulletin No. 6, August, 1889. The other plots, as was most of the corn in this vicinity, were also affected but not to such an extent as those noted. Very early varieties of corn were affected more than the later ones that were planted at the same time. Some of the early corn grown by the market gardeners was entirely destroyed. The corn first assumes a yellowish appearance, stops growing and then in bad cases, rots off at the ground."

Burrill in 1879 (4) had demonstrated that bacteria could produce disease in plants. Stewart began his work as a plant pathologist on Long

Island in 1895. During 1895, 1896 and 1897 (37), he found this disease doing considerable damage in the market gardens there. Losses of 20 to 40 per cent were frequent but in most cases the loss was so slight as to pass unnoticed by the farmers, although the disease could be found in almost any field of early sweet corn on the Island.

Erwin F. Smith (31), who had come into the Department of Agriculture in 1886 (40), found the disease in two fields of common field corn in southwest Michigan in 1898 (30, 31). Halsted, beginning the study of plant diseases in New Jersey in 1899 (15), reported bacterial wilt. In 1903 it was found for the first time in Maryland and the District of Columbia (31). In 1908 wilt was found in a garden in Falls Church, Va. Selby in 1910 (29) reported a serious bacterial disease of sweet corn in Ohio but did not identify it as the bacterial wilt disease. Wilt was reported to the Plant Disease Survey from California in 1909. Mrs. Enlows found it in West Virginia in 1913 (31) and Garman in Kentucky in 1916 (14). Apparently the disease was widely distributed as Stewart and Smith had suggested and when Rand began his work in 1918 and made the first systematic search for it he reported bacterial wilt from most of the Central and Southern States (24)—from Connecticut, Massachusetts and southern New York to Georgia and westward through the Corn Belt to Iowa, Missouri, Oklahoma and New Mexico. Although he searched for several years he did not find the disease in North Dakota, Minnesota, Wisconsin, Michigan, northern New York, Vermont, New Hampshire or Maine (27).

During most years the disease was not of sufficient importance in any of the states to attract attention but in certain years it caused heavy losses. By 1922 and 1923 canners in Maryland were forced by heavy losses in early susceptible varieties of sweet corn to grow only the later maturing resistant sorts (27). During the five years from 1929 to 1933 the disease became more abundant and destructive than at any time in its history. Susceptible varieties in market gardens in Illinois, were in many cases a complete loss. The disease spread farther north and caused losses in Maine [6] and southern Ontario [1] and was found in New Hampshire[5], central New York[7] (7) and Michigan[2] (33).

Increased cultivation of susceptible varieties of sweet corn has undoubtedly been one of the factors contributing to this increase in amount and distribution of bacterial wilt. The study of other factors during the past 35 years has added to our knowledge of dissemination of the disease. Early investigators were concerned with seed and soil transmission.

Stewart (37) felt that the organism entered the underground parts of the plant from the soil or seed but his experiments were carried on out of doors in a region where the disease was prevalent, his checks became contaminated and his results were not conclusive.

Smith's (31) observations in Michigan in 1898 led him to believe that infection took place through above-ground parts of the plants. He found lesions starting near the tips of the leaves and concluded that the bacteria gained entrance through the water pores or stomata. The results of experiments on soil transmission, conducted by Rand and Cash (27) in 1920-1923, are summarized in table 1.

Rand found that in the field the percentages of wilt in several tests were approximately the same in inoculated and control plots. In the

TABLE 1. *Summary of results of experiments on soil transmission conducted by Rand and Cash in 1920—1923*

Location	Varieties	Number of plants		Method of Inoculation	Average percentage of wilt	
		Inoculated	Controls		Inoculated	Controls
Field 5 tests	Susceptible variety of sweet corn	About 1400	About 1400	Diseased corn stalks planted with seed	41.0	38.0
2 tests	Do	1600	Adequate	Diseased stalks buried in the fall, seed planted in the spring	24.0	26.0
2 tests	Do	183	183	Soil inoculated with water suspension of bacteria at planting time	70.0	62.0
1 test	Do	80	Adequate	Seed soaked in bacterial suspension before planting	74.0	64.0
Field In cloth covered cages 2 tests	Do	17		Soil inoculated Seed inoculated (As above)	0 0	
Greenhouse 20 tests	Do	2500		Soil inoculated Seed inoculated (As above) Except by inoculation of soil at time of root pruning	0 0	

greenhouse and in cloth covered cages, where insects were excluded, not a single case of wilt developed with the exception noted. He concluded that his experiments gave no evidence of any transmission through the soil but did give definite evidence against it.

Reddy in 1921 (28) reported that he obtained no cases of natural infection from soil which immediately before had produced an artificially infected crop.

Thomas (39) working in the greenhouse in Ohio in 1922-23, planted Whipple's Early sweet corn in a bed in which disease-free plants had been grown and also in another bed in which diseased plants had been grown and then chopped up and mixed with the soil. He obtained 3 per cent infection in the uninoculated and 26 per cent in the inoculated bed. White flies and winged aphids were present on the corn plants.

Clinton and Singleton (8) carried on a soil transmission test in the open field, on land not recently in corn, in 1933 near New Haven, Conn Infected plant tissue, kept indoors overwinter, was mixed with the soil.

Just before tasseling the percentages of infected plants were 26 for the inoculated row and 29 for the uninoculated.

In 1933 Ivanoff (17) reported the results of experiments in the laboratory and greenhouse testing the penetration of wounded and unwounded roots of corn seedlings by *Aplanobacter stewarti*. Bacterial suspensions were placed on the tips of 50 surface sterilized kernels which were then grown for 2 weeks on agar. No disease symptoms developed and bacteria were not found inside the tissues. Part of the roots were then wounded and 4 days later these plants showed characteristic stripes; 42 seeds soaked in bacterial suspensions over night and grown for 3 weeks after germination on agar showed no signs of infection. Four-day seedlings were painted with bacterial suspensions, a part were wounded and all were planted in autoclaved soil. One week after planting some of the wounded plants were diseased and all of the unwounded plants were healthy. In a fourth test over 500 plants, 2 to 3 weeks old, were planted in boxes in steamed soil inoculated with bacterial suspensions. The roots of part of the plants were wounded and one month later 18-100 per cent of these plants were diseased. Three per cent of the plants with unwounded roots became diseased, which he attributed to accidental wounding.

The evidence for soil transmission of bacterial wilt is apparently negative except for Thomas' results and they may have been complicated by insects.

One of the most interesting cases of circumstantial evidence of seed dissemination of wilt is reported by Erwin F. Smith (31). In 1902 an Ohio farmer grew sweet corn under contract for a Philadelphia seed company. In November the farmer wrote the seed company that he would be able to deliver only 20 per cent of the quantity of seed promised because the corn had rotted. This seed formed part of the Congressional Free Seed Distribution of 1903. Some of this seed planted in a garden in Takoma Park, Md., came up badly and Dr. Smith found one plant showing typical symptoms of wilt. Some of the same seed was planted south of the Monument grounds in the District of Columbia. In July he found 15 per cent of this variety diseased and a month later 80 per cent. Many other varieties were more or less diseased. More of this same seed planted in July, across the river at Arlington Farm, Va., showed 16 per cent wilt at the end of the season. These were the first reports of the disease from Maryland and the District of Columbia. Something was apparently spreading the disease on the Monument grounds; and since Smith's inoculation plots of 1902 were only a quarter of a mile away from the Monument grounds it is possible the disease in this plot did not all originate from seed. In 1908 the disease was found in Falls Church, Va., and the seed was again traced to the same grower in Ohio. Smith planted some of this seed in the greenhouse and some out of doors in pots. Nine per cent of the plants from unselected seed and 9.3 per cent from selected bad seed in the greenhouse and 0.5 per cent in pots out of doors became diseased. The plants in pots were crowded and stunted. Smith attempted to isolate the wilt organism from the surface of this seed but did not succeed. Rand and Cash also failed to isolate *Aplanobacter stewarti* from the surface of seed. Smith did however demonstrate the presence of the organism inside the seed in stained

sections. In 1921 Rand and Cash (24) isolated the organism from the endosperm of seed, grown on diseased plants, 5 months after harvest. Ivanoff (17) has obtained positive results from isolations and also published photomicrographs showing the organism in the vascular tissue, chalazal region, and endosperm of the seed.

Rand and Cash (27) carried on a number of seed transmission tests in the greenhouse and field, the results of which are summarized in table 2.

In the greenhouse under controlled conditions where insects were absent they obtained 2 to 13 per cent infected plants. In the open field the percentages were high and varied for susceptible varieties regardless

TABLE 2. *Summary of results obtained by Rand and Cash on seed transmission tests in greenhouse and field*

Location	Number or quantity of seeds	Source	Infected plants	
			Number	Percentage
Field tests				
5		From healthy plants		43.0
		From diseased plants		56.0
1		Open market		3.0
		Diseased plants		6.0
1		Open market		72.0
		Maine and Michigan		83.0
		Infected plants		78.0
3		Open market		48.0
		Maine and Michigan		54.0
10		Diseased plants $\begin{cases} 1 \text{ year old} \\ 2 \quad " \quad " \\ 3 \quad " \quad " \end{cases}$		17.0 / 24.0 / 18.0
		Maryland healthy plants $\begin{cases} \text{Golden Bantam} \\ \text{First of All} \\ \text{Premo} \\ \text{Peep O' Day} \\ \text{Golden Cream} \end{cases}$		71.0 / 59.0 / 40.0 / 27.0 / 27.0
		Maine Golden Bantam		92.0
		Michigan Golden Bantam		86.0
		Maine—other varieties		96.0
	470 lots	Seedsmen in all sections of the United States (2 early varieties) (2 late varieties)		48.0 / 5.0
Greenhouse tests				
1	23	Badly diseased ear	3	13.0
1	54	Several badly diseased ears	1	2.0
1	2 quarts	Badly diseased crop		2.0
	Hundreds	Open market	0	0

of whether the seed came from healthy or diseased plants or from sections where the disease was prevalent or absent. They concluded that "transmission by seed is a serious factor, probably the only factor, in introducing bacterial wilt of corn into new localities; and inroads from this source alone may be considerable." However, "once the disease becomes prevalent in a locality, the planting of resistant or susceptible varieties or strains is far more important in determining the ultimate damage to the crop from wilt, than is the origin of the seed from healthy or wilt diseased plants."

Rand and Cash (24, 25, 26, 27) also found that moisture and soil were important factors in determining the amount of infection from diseased seed. Much more wilt developed when rain was plentiful at planting time than during dry periods. In one field where seed was planted across three types of soil they obtained 21 per cent wilt on poor soil, 27 per cent wilt in the transition section between, and 41 per cent wilt on the rich soil.

Thomas (39) reports that in 1922-23 he grew Whipple's Early sweet corn in the greenhouse, obtaining 85.0 per cent infection from diseased seed and no infection from clean seed.

Rand's observations and experiments during 1918 and 1919 led him to the conclusion that seed and soil transmission of the disease could not account for either the primary infections developing early in the season or the midseason spread of the disease in the field. In the greenhouse no infection resulted from soil inoculation and wilt from diseased seed averaged only about 2 to 13 per cent. In the open field abundant infection resulted regardless of whether the seed was clean or diseased or was planted on infested or clean soil. Seed from Maine and Michigan where the disease did not occur produced plants with higher percentages of disease than plants from seed grown where the disease was prevalent. He also observed that many of the infections started from insect feeding injuries on the outer halves of the leaves and that at harvest time many plants showed infection on the upper parts of the plant and none in the lower part. These facts all pointed to insects as the principal means of dissemination of bacterial wilt (27). In 1920 he began his cage experiments.

The results of insect transmission tests by Rand and Cash (26, 27) with the 12 spotted cucumber beetle *Diabrotica duodecimpunctata* are summarized in table 3.

Only rarely in the field or in direct tests in cages was wilt observed to start from points gnawed by these insects. But small channels at the base of the stems were frequently observed in insect cages. They concluded that very little secondary leaf infection came from direct transfer by feeding of these insects but that some of the primary basal infection might come from their larval borings at the crown.

Numerous cases of secondary leaf infection in the field were observed to have started apparently from injuries by flea beetles.

Results of tests with these beetles (25, 26, 27) are given in table 4.

These direct tests confirmed his field observations that at least in Maryland the great bulk of secondary infection—late spring and summer spread of the disease—is brought about by direct transfer by flea beetles.

In 1932 Ivanoff (16) reported finding insect larvae feeding on the roots and bases of stems of diseased corn plants. He isolated *A. stewarti*

TABLE 3. *Summary of results of tests by Rand and Cash on insect transmission with the Diabrotica duodecimpunctata*

Treatment	No. of plants	Infected plants	
		Number	Percentage
A. Direct feeding tests in cloth covered cages—			
4-6 bettles from diseased plants introduced into cages	389 182	6 0	1.5 0
B. Direct inoculations with intestinal contents pricked into the leaves—			
In field 3 tests in cloth cages	74	15	21.0
In greenhouse 26 beetles from diseased corn fed 3 weeks on healthy plants			19.0
16 beetles fed 5 days on diseased plants then 1 month on healthy plants			25.0

from the larvae and from the plants. Ten days after the larvae had been placed on the roots of healthy plants wilt developed in 9 plants. Two of the larvae had died—6 larvae from healthy plants produced no infection. This experiment was repeated four times with similar results. In 1933 (17) he placed white grubs, the larval stage of Phyllophaga spp., in the boxes in which plants were growing in artificially infested soil. Fifty per cent of these plants became infected. In three boxes without grubs no disease appeared.

To this accumulating evidence that insects play an important part in the dissemination of bacterial wilt during the late spring and summer, work at Arlington Farm, Virginia (9), during the past year has contributed the additional information that the same beetle which Rand found carrying the bacteria to high percentages of corn plants is also probably responsible for overwintering of the organism and for a large part of the primary infections at the beginning of the season.

Overwintered adults of the flea beetle *Chaetocnema pulicaria* Melsh were collected from orchard grass and alfalfa during April, 1934. Beetles

PLATE I

Leaf of Golden Bantam hybrid showing flea beetle injuries at tip of leaf and beginning wilt lesions. Photo July 26, 1934.

PLATE I

PLATE II

Leaf of Golden Bantam sweet corn showing flea beetle feeding injuries and beginning bacterial wilt lesions X 10. Sept. 6, 1934.

PLATE II

PLATE III

Natural infection with *Aplanobacter stewarti* on leaves of *Euchlaena mexicana* grown at Lanham, Md., from Florida seed. Collected Sept. 5, 1934.

PLATE III

PLATE IV

Plants of *Euchlaena mexicana* from Florida seed.

Pot A.   Inoculated Oct. 2, 1934, with isolation 52-13 from *Chaetocnema pulicaria*. Photographed Oct. 31, 1934.

Pot B.   Uninoculated.

PLATE IV
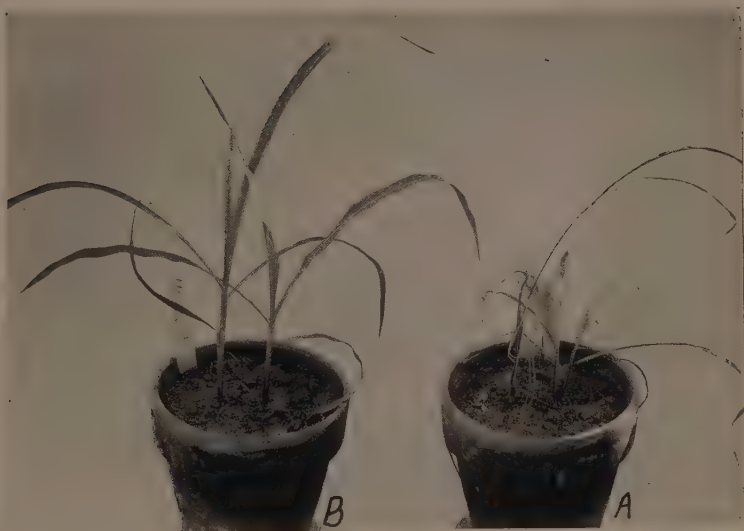
TABLE 4. *Results obtained by Rand and Cash (25, 26, 27) from tests with beetles*

No. of tests	No. of cages	Insect	Treatment	Percentage of infected plants
7	16 (4 plants to a cage)	*Chaetocnema pulicaria*	Fed 24-48 hours on diseased plants before introduced into cage	
1				100.0
2				100.0
3				100.0
4				100.0
5				57.0
6				60.0
7				87.0
Controls	441		No insects	0
Several	16	*Ch. denticu-lata* (12-50 to a cage)	Fed on diseased plants before in-troduction into cage	83.0
Controls	441		No insects	0 (except in 5 torn cages)

collected on four different dates were surface sterilized, and then crushed in sterile beef peptone broth. Platings from this broth gave heavy seedings of *A. stewarti* in practically pure culture from each of the four collections. The organisms isolated produced typical wilt infection on corn plants in the greenhouse. When beetles from the same collections were permitted to feed for several days on healthy corn plants in the greenhouse, typical wilt lesions developed and *A. stewarti* was reisolated. Isolations from 175 single individuals indicated that the wilt organism was present in abundance in approximately 19 per cent of the beetles.

In the light of these facts concerning insect transmission it is inter-esting to recall the evidence that flea beetles have probably been the chief means of dissemination since the first reports of bacterial wilt. The wilting of corn plants attacked by *C. pulicaria* in Illinois in 1891 (12) and the killing of the terminal part of the leaf beyond the feeding injuries are very suggestive of this disease. Smith's observations in Michigan in 1898 (30, 31) that wilt lesions occurred on the tips of the leaves are quite in accord with the fact that flea beetles feed largely on the outer halves of the leaves (Pls. 1 and 2). Stewart (37), Smith (31), and Rand and Cash (25, 26, 27) all found that experiments carried on in the open field were of little or no value because of the abundance of infection occurring on checks as well as inoculated plants, and on all susceptible varieties regardless of source or condition of seed.

That a succession of mild winters paved the way for the epidemic of bacterial wilt in Illinois in 1932 is noted in the (3) Kane County Farmer for November, 1932.

The relation of winter temperatures to the distribution and abun-dance of bacterial wilt has been discussed recently by Stevens (34, 35,

36). He points out that when the average winter temperatures are high wilt has shown a tendency to increase in visa versa. The unusual abundance and distribution of wilt from 1929 to 1933 followed a series of mild winters and the amount and distribution of wilt in 1934 was apparently much reduced following the low temperatures of the previous winter. The winter of 1889-1890 in Illinois was unusually warm. The temperatures for December, January, and February at Springfield, Ill., averaged 8.5 degrees above normal and those of the same three months for 1890-1891 averaged 3 degrees above normal.

Rand and Cash found flea beetles abundant in the sections where wilt is prevalent but did not find them in Maine (27). The possible relation of winter temperatures to the numbers and distribution of infested flea beetles is suggested by these facts.

The subject of alternate hosts for bacterial wilt has apparently received little attention. Stewart (37) reported negative results from the inoculation of oats and of teosinte *(Euchlaena mexicana)*. During the past summer natural infection with *A. stewarti* on teosinte was found in the field at Lanham, Md. (Pl. III). The lesions were similar to the leaf lesions on corn but more limited than on susceptible corn varieties. Isolations from these leaf lesions gave pure cultures of a yellow bacterium which produced typical wilt symptoms on Golden Bantam sweet corn in the greenhouse. A culture of *A. stewarti* isolated from *C. pulicaria* produced long wilt lesions on teosinte leaves in the greenhouse and killed the plants (Pl. IV). It would be of considerable interest to determine whether or not the disease occurs on teosinte in its natural habitat in Mexico.

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PRELIMINARY STUDIES ON THE EFFECT OF FILTRATES FROM CULTURES OF DIPLODIA ZEAE UPON SEEDLING BLIGHT OF MAIZE[1]

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Many plant pathogens have been reported to produce materials toxic either to themselves or to their hosts by various investigators. In the course of experimentation upon the relationship of the dry rot fungus, *Diplodia zeae* (Schw.) Lev., to its host, the corn plant, similar reactions have been observed. The amount of seedling blight in plants from severely infected seeds may be decreased materially by soaking the seed, prior to planting, in filtered aqueous extracts from aged cultures. Since the treatment of an infected seed subjects both the plant and the fungus to the action of the filtrate any of the four possible reactions which have been reported in the literature may occur.

The failure of a fungus or bacterium to grow in aged culture has been very commonly attributed to an accumulation of autogenic products, the so-called "staling products." More recently, developmental failures in bacteria have been attributed to bacteriophage activity. Mallman and Hemstreet (6) in 1924 isolated a principle from decaying cabbage which was lytic to the causal agent, a fluorescent bacterium, and to *Bacillus carotovorus* Jones. Similar bacteriophages have been isolated by Coons and Kotila (4) for *B. carotovorus* and *Pseudomonas tumefaciens* Smith and Town., by Anderson (1) for *Pseudomonas pruni* E. F. S. and by Muncie and Patel (7) for *Ps. tumefaciens*. The presence of a similar agent in fungi is yet to be demonstrated.

A toxic reaction to the host is frequently reported, especially by those interested in the wilt diseases induced by vascular parasites, particularly of the genus Fusarium. Schaffnit and Ludtke (9) working with *Fusarium lycopersici* Sacc. and *F. vasinfectum* Atk. concluded that diamino acids were responsible for the wilting caused by filtrates from giant cultures on mixed grain. Toxins produced by plant parasites also have been described by Clayton (3), Wolf (10), Ludtke and Achmed (5), Rosen (8), and others. In apposition to this harmful effect, Arnaudi (2) reports that when the seed of Berkley tobacco is germinated in an aqueous suspension of attenuated mycelium of *Thielaviopsis basicola*, the resistance to subsequent infection by this organism is increased. He describes the treatment as a vaccination and claims that immunity is maintained for about two months.

The effect of the cultural filtrate upon the relationship existing between host and parasite may be readily studied in *Diplodia zeae* from corn. The organism can be grown on any one of 20 or more natural and synthetic media. It will use a wide range of carbon compounds (levulose,

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xylose, dextrose, galactose, lactose, inulin, mannose, maltose, sucrose, starch, cellulose, citric acid, and even dilute ethyl alcohol) in agar plates or in a nutrient solution such as Czapek's. The growth is rapid and the fungus matures in about three weeks at 30° C., readily forming a heavy mat on the surface of nutrient solutions.

Diplodia zeae is parasitic on all parts of the corn plant. Stem and leaf sheaths are infected late in the growing season by wind-borne spores, the underground parts of the plant by active mycelium in the soil and the mesocotyl of the seedling from seed-borne infection. The severity of the mesocotyl infection depends upon the degree of infection of the seed which can be determined by a germination test. The kernels from an ear inoculated in the early dough stage exhibit all degrees of invasion from destruction of the embryo to mere harboring of the fungus in the seed tip. Since the effectiveness of any protective measure for the plant could be accurately determined in seed with a weakened embryo, all studies were directed toward seedling infection. Data on the effect of filtrates from cultures on parasitism of the pathogene are presented in the following pages.

EXPERIMENTAL RESULTS

Ears of corn artificially inoculated with *Diplodia zeae* through the shank while still in the field were tested on a germinator and the kernels, ranging from dead at the butt to disease-free at the tip, were carefully separated into four or five classes. In all the tables these classes are designated as being derived from infection zones numbered one to four or five from the butt end. The seed from each zone was divided into two lots, one of which was soaked for 12 to 24 hours in distilled water or in filtered media and the other in a filtered extract of the fungous culture.

In the first experiment a 138-day-old culture of *Diplodia zeae* on whole oats was ground in a Wiley mill, soaked for several hours in distilled water and filtered through a Berkfeld "W" cylinder into a sterile flask. Seeds from four zones from two different ears were soaked in this

TABLE 1. *Increased germination and stand of corn from seed showing different degrees of infection by Diplodia zeae when soaked in filtrate from a culture of the same organism*

Seed from infection zone	Seed in	Seed from ear A			Seed from ear B		
		Number seeds	Stand at		Number seeds	Stand at	
			7 days	20 days		7 days	20 days
Four	Filtrate	48	46	46	50	48	48
Four	Water	48	47	47	50	49	49
Three	Filtrate	12	10	10	19	19	19
Three	Water	12	12	12	19	17	17
Two	Filtrate	12	11	12	25	17	21
Two	Water	12	12	11	25	6	4
One	Filtrate	20	18	18	25	11	14
One	Water	20	5	3	25	2	1

filtrate and in distilled water for 16 hours under identical conditions. The seeds were planted in compost under greenhouse conditions, and the number of seedlings which emerged and survived was recorded. These data are presented in table 1.

These data were so conclusive in showing that a filtrate from the culture could apparently shift the balance of infection in favor of the plant that the experiment was repeated. The same technique was applied, the filters and all glassware being thoroughly cleaned. The same general conditions held when cultures of the same age on either oats or barley were used. Where the filtrate was used a larger number of seedlings escaped seedling blight.

A further test of the significance of the difference in the stands from seed soaked in the filtrate and water was made upon a heterogenous mixture of seed showing all degrees of infection. The test showed 25 per cent of the seeds to be viable. The majority of these were disease-free. The seeds were divided into 16 lots of 20 seeds each and eight lots were soaked in the filtered extract from giant oat culture and the remainder in water for 17 hours. Seven of the eight replications showed an increase over the checks. The eighth replication was exposed to a cold wind through a broken pane of glass, so that the seedlings were chilled before emergence. The results presented in table 2 show a mean difference of 4.4 plants with a standard deviation of 1.03 plants for each group of 20 seeds; so the differences are highly significant.

TABLE 2. *Increased stand in eight replications of corn grown from seed infected by Diplodia zeae which were soaked in culture filtrate prior to planting*

Treatment of seed	No. seed planted	Number plants		Difference due to treatment
		Emerging	Surviving	
Water	20	4	2	
Filtrate	20	10	10	+ 8
Water	20	6	6	
Filtrate	20	9	9	+ 3
Water	20	7	6	
Filtrate	20	10	9	+ 3
Water	20	3	3	
Filtrate	20	11	10	+ 7
Water	20	5	4	
Filtrate	20	11	11	+ 7
Water	20	8	6	
Filtrate	20	9	9	+ 3
Water	20	4	4	
Filtrate	20	9	8	+ 4
Water	20	3	3	
Filtrate	20	4	3	0
Summary: Water	160		34	
Filtrate	160		69	+35

TABLE 3. *Effectiveness of filtrate from 300-day-old culture of Diplodia zeae grown on Czapek's solution in increasing the stand of corn from infected seed*

Seed from infection zone	Seed soaked in	Seed from ear A			Seed from ear B		
		No. seed	Emergence	Stand	No. seed	Emergence	Stand
Four	Water	20	20	20	25	25	25
Four	Filtrate	20	20	20	25	25	25
Three	Water	20	14	11	20	20	20
Three	Filtrate	20	16	16	20	20	20
Two	Water	14	3	2	10	7	6
Two	Filtrate	14	4	3	10	10	10
One	Water	20	0	0	20	2	2
One	Filtrate	20	1	1	20	7	7

The results from soaking seed in a filtrate which obviously contains much foreign plant material might be attributed to the protein or other ingredients derived from the oats. To eliminate foreign plant parts, an experiment similar to the first was run, using a filtrate from a 300-day-old culture grown on Czapek's solution with a sucrose base. The results of this test, given in table 3, show a consistent difference in stand where the seed was soaked in the filtrate, although the percentage difference was not great. Repetition of this experiment confirmed the results.

Since all the cultures used above were more than four months old, the effect of age of the culture was next studied. A more desirable experimental procedure was followed at the same time. For each flask of sterilized oats which was inoculated with *Diplodia zeae* an uninoculated flask was held as a check. Whenever the culture was extracted, the checks were similarly extracted and filtered and the two solutions were used simultaneously on infected seed. The cultures were extracted at three, five and six weeks of age and lots of ten seeds each from the same ears were soaked for 20 hours. A small number of seeds was used so that the seed for each test might be from the same source.

The results shown in table 4 indicate that at three weeks old the filtrate from the culture was actually more harmful than beneficial; while two weeks later the difference in stand from the infected seed indicated that the filtrate was beneficial. The inconsistency of the results from a three-weeks-old culture was checked by starting new cultures and repeating the experiment. Since the same results were obtained from this repetition, the younger culture on oats apparently produced some material injurious to the germinating seed. The indifferent results from the use of the six-weeks-old culture were later explained upon the temperature relationships set forth below. This experiment was set out in the greenhouse during the first unseasonably warm period of the 1934 spring.

The apparent inconsistency of the results from filtrates of the actively growing culture three weeks old, and the more mature culture five weeks old, was explained upon the basis that two products were formed. One of these apparently retards the development of the seed; the other hinders the parasitic invasion of the fungus.

TABLE 4. *The effect of age of culture on effectiveness of filtrate in preventing seedling blight*

Seed from infection zone	Ear no.	Seed soaked in extract from	No. seed	Number that emerged with culture of various ages		
				3-weeks-old	5-weeks-old	6-weeks-old
Four	9	Culture	10	10	10	10
Four	9	Check	10	10	10	10
Three	9	Culture	10	8	10	7
Three	9	Check	10	8	9	5
Two	9	Culture	10	2	6	3
Two	9	Check	10	8	2	3
One	9	Culture	10	0	2	1
One	9	Check	10	2	1	0
Two	1	Culture	10	3	8	3
Two	1	Check	10	6	6	4

Previously it had been noted that three-weeks-old plants grown from seed soaked in filtrate from aged cultures on Czapek's solution and sucrose suffered a severe shock within six hours after transferring to a similar, diluted filtrate; while those transferred to filtrate from uninoculated media remained turgid. The plants in the filtrate recovered, but within three days assumed a yellowish hue while the checks remained normal.

Such a reaction was proven later to be not an anaphylactic shock, but rather a reaction to a volatile toxic component of the filtrate which may be distilled off in the first few cubic centimeters of the distillate. The residue remaining in the flask is beneficial in promoting germination and growth of infected seeds. This active residue is not destroyed by a temperature of 100°C. for one hour.

The filtrate treatment of seeds was found to be more effective when the seeds were germinated at low temperatures. During the summer months, several tests gave indifferent results. When placed in a refrigerator at 16°C. until emergence, the treated seed showed an increase over their checks. The effectiveness of the treatment at 16°C. and 26°C. is shown in table 5. The three ears used in these two experiments were tested in the usual way. Ear A had severe infection with intergrading zones ranging from moderate infection to dead with no disease-free or lightly infected seed. At the higher temperatures the plants apparently outgrew the infection and became established before seedling blight could occur; as a result the filtrate gave indifferent results. However, at lower temperatures where most of the seedlings emerge, but die before establishment of the secondary roots, the filtrate treatment was capable of saving many of them.

The filtrate does not seem to stimulate growth of the plant in a manner analogous to an increase in temperature. Obviously, the filtrate could do one of three things to increase the stand at 16°C. It could stimulate growth of the plant, inhibit the fungus, or induce resistance in the plant. The rates of emergence and growth of seedlings from seeds soaked in culture

TABLE 5. *Influence of temperature upon effectiveness of culture filtrate as a treatment of corn seed infested with Diplodia zeae*

Seed from infection zone	Seed soaked in	Temperature germination	First Experiment					
			Ear A		Ear B		Ear C	
			No. seed	Emergence	No. seed	Emergence	No. seed	Emergence
		Deg. C.						
Five	Water	16			10	10	15	15
Five	Filtrate	16			10	10	15	13
Five	Water	26			10	10	15	15
Five	Filtrate	26			10	10	15	13
Four	Water	16			10	9	15	10
Four	Filtrate	16			10	10	15	14
Four	Water	26			10	8	15	9
Four	Filtrate	26			10	8	15	13
Three	Water	16	10	5	10	8	15	3
Three	Filtrate	16	10	10	10	10	15	7
Three	Water	26	10	7	10	9	15	7
Three	Filtrate ·	26	10	9	10	9	15	7
Two	Water	16	10	1	10	1	15	0
Two	Filtrate	16	10	7	10	9	15	0
Two	Water	26	10	4	10	6	15	2
Two	Filtrate	26	10	4	10	7	15	3
One	Water	16	10	0	10	0	15	0
One	Filtrate	16	10	3	10	1	15	0
One	Water	26	10	1	10	2	15	1
One	Filtrate	26	10	3	10	2	15	0

and media filtrates are very similar. If there is any difference in the time required for emergence between the two lots of seeds, those soaked in filtrates from cultures are slower as is shown by the severely infected lots in table 1.

The filtrate does not entirely prevent the growth of the fungus on agar plates. Media impregnated with the filtrate (50 cc. to 150 cc. of corn meal agar) supported nearly as abundant growth as corn meal agar and distilled water. The aerial growth was heavier and the lateral growth about 20 per cent less than that in the check plates. Infected seeds soaked for long periods in the filtrate will show a growth of *Diplodia zeae* into the filtrate itself.

SUMMARY

Corn seedlings from seeds severely infected by *Diplodia zeae* suffer less from seedling blight when the seed has been immersed in a cultural filtrate of the pathogene prior to planting.

The ability of the filtrate to prevent seedling blight seems to be independent of the character of the media upon which the fungus is grown.

The pathogene must have passed the active growing period before the cultural filtrate becomes effective.

The influence of the filtrate on emergence was pronounced at 16°C.

where the plant was at a disadvantage with the pathogene, but was very slight at higher temperatures.

The culture filtrate does not prevent seedling blight by stimulating the plant to abnormally rapid growth or by absolutely preventing growth of the pathogene.

The filtrate generally contains a mixture of materials. The beneficial component is thermostable and non-volatile so it can be partially purified and concentrated by distilling off the volatile fraction, which is slightly toxic to the plant.

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THE ONTOGENY OF THE MAIZE PLANT—THE EARLY DIFFERENTIATION OF STEM AND ROOT STRUCTURES AND THEIR MORPHOLOGICAL RELATIONSHIPS

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This paper is a partial report of the investigations of the morphological development of the maize plant from the date of planting to maturity.

That there is a correlation of the more obvious morphological features of the maize plant and the yield is unquestionable. It is a general observation that the large ears are not borne on small or weak stalks. Although studies on the relation of yield to differences in leaf area, height of plant, weight of plant, diameter of stalk, number and length of internodes and in various ear and kernel characters have not in general resulted in definite conclusions, it is still a reasonable expectation that if differences in environmental influences were eliminated there would be discovered a positive correlation of yield and the morphological features that especially have to do with the absorption, conduction, and food-manufacture of the plant.

Miller's (14) comparative studies of the root systems and leaf areas of maize and of the sorghums show that the ratio of the weight of the root system to the leaf area in maize is about half the ratio in the drought resistant sorghums, and that the difference is due to the proportionately greater leaf area in maize. This fact is evidence in favor of the prevalent opinion that the character of the root system of a maize plant is a major factor in determining its development and yield.

That the development of the maize plant is much influenced by competition has been shown by a number of investigations, notably by Morrow and Gardner (16), Sheppard and Ten Eyck (18), Kiesselbach (12), Brown and Garrison (3), Bryan (4), Hughes (10), Williams (20), Brewbaker and Immer (1), Eisele (7), and others.

Koehler's (13) work shows that competition inhibits the normal development of maize plants. According to Eisele (7) competition has a marked influence on the leaf area, size of the stem, and the number of roots. He found that in hills of five plants, the stems were less than half the size of the stems of plants grown singly. Eisele noted also that the inhibitory effect of competition on development was pronounced in the stem before it was recognizable in the leaves. Hershey (9) found that both competition and shading of maize plants resulted in a marked reduction in the size of the stem and also in the number of vascular bundles in the stem.

Most of the investigations of correlations in maize have had for their object the discovery of features related to yield, that can be used as a criterion in seed corn selection. They have been concerned chiefly with ear and kernel characters, but some have included leaf area, height and diameter of stems, number of nodes, and length of internodes.

Among the investigations of correlation in maize involving morphological features are those of Brigham (2), Ewing (8), Nielsen (17), Montgomery (15), Jenkins (11) and Collins (6). Both Brigham and Ewing reported a significant positive correlation between diameter of stems and yield. Brigham found some positive correlation of the number of internodes with yield. Jenkins' (11) study of inbred parents and crossbred progeny disclosed a significant positive correlation of yield with the height and number of nodes of the parents.

The investigations by Hershey (9) have disclosed that in the varieties included in this article there are three rather distinct periods in the morphological development of the plant. The first period, which was covered by the first 30 to 40 days and quite generally by the first 35 days after planting, was devoted to the differentiation of structures, such as the nodes, internodes, leaves, axillary buds, vascular bundles, roots, and tassel. At the end of this period almost all the structures the plant possessed at maturity were present, although most of them were in the primordial stage of development. This first or formative period was immediately followed by a period in which the emphasis was on the completion of the development of the structures present. This second period was covered by the 35 to 40 days following the formative period, and was characterized by a very rapid elongation of the stem axis and the completion of the development of the structures formed in the preceding period. The plants attained their mature size during this period. The third period included pollination, fertilization, and the development and maturing of the kernels.

This discussion is confined chiefly to the following morphological features of the formative period and their significance on the final achievement of the maize plant: the status of the development of the stem axis at the end of the formative period, both as to its external structures—namely, the nodes, leaves, axillary buds and tassel, and as to its vascular bundles; the development and nature of the permanent roots; the relation of the number of bundles to the size of the stem axis; the relation of the number of nodes forming soil roots, of the number of roots in the upper whorl of soil roots, and of the average cross-sectional area of the roots in each whorl, to the diameter or cross-sectional area of the lower internodes of the stem axis; and the relation of the number and total cross-sectional area of the tracheal tubes in the roots to the average cross-sectional area of the roots.

MATERIALS AND METHODS

In 1927 and 1928 the investigations were confined to common strains of Reid's Yellow Dent and Stowell's Evergreen corn. In 1929 and the following years there were included five yellow dents, Stein's Reid, McCulloch's Reid, Osterland's Reid, Golden King, and a hybrid (274 x 276). The hybrid was produced by Dr. M. T. Jenkins and was a double cross between an F_1 of Lancaster 325A x Iodent 175A and an F_1 of Black's 245B x McCulloch's 401A.

The plants were grown in field plots where the plantings were carefully supervised. In the study of the correlation of the number of vascular bundles and the diameters or cross-sectional areas of the lower internodes plants from both the field and the greenhouse were employed.

In following the anatomical changes during the first or formative period of development, paraffin sections were necessary up to 30 days after planting. The material was collected at intervals ranging from two to seven days according to the stage of the development of the plant and the nature of the structures under consideration. Both Bouin's and F. A. A. killing fluids were satisfactory. To hasten the penetration of the killing fluids, the leaves were removed and in the older stages lengthwise slabs were cut from one or more sides of the stem. Delafield's haematoxylin and haemalum in combination with safranin were satisfactory stains. In the study of the later anatomical features, such as the number and the status of the development of the vascular bundles in the stem axis and the vascular anatomy of the roots, free-hand sections stained with safranin and haematoxylin were satisfactory.

The age of plants was reckoned from date of planting. Height of plants was determined with leaves upstretched.

THE DEVELOPMENT OF STEM STRUCTURES DURING THE FORMATIVE PERIOD

DEVELOPMENT OF THE STEM AXIS

Previous to germination, the embryo of maize consists of a relatively large scutellum and a small axis terminating in the radicle at one end and in the plumule at the other. The apex of the plumular end is the conical meristem which by the addition of new cells through cell division provides for the formation during subsequent growth, of additional nodes, internodes, leaves, axillary buds, and finally the tassel (Pl. I, fig. 1). The number of nodes and consequently the number of leaves of a maize plant depends upon the extent to which the meristematic tip functions as a meristem. In the varieties included in these investigations the plumular end of the embryonic axis possessed six to nine nodes previous to germination. At each node, except the lowermost which bears the coleoptile, a primordium of a leaf was present and at some of the older nodes primordia of axillary buds were recognizable.

During the period of 30 to 40 days following planting the number of nodes present in the embryonic axis was increased to the number present in the mature plants, which was 20 to 24 in most of the plants investigated. There were added, therefore, to the embryonic axis during the formative period, nodes, internodes, and leaves ranging in number from approximately 11 to 18. This fact emphasizes the importance of the meristematic activity of the tip meristem during the plant's early growth in determining the number of nodes and leaves of the plant. The addition of new nodes and accompanying structures terminated with the transformation of the meristematic tip into the primordium of the tassel. In the Stowell's Evergreen and in the yellow dents included, the transformation of the tip meristem into the tassel primordium occurred usually previous to the thirty-fifth day after the date of planting (Pl. I, fig. 2). At this time the stem axis was seldom more than three inches in length although the plants were two feet or more in height (Pl. I, fig. 4).

In his investigations of the vascular anatomy of the first and second internodes above the soil roots, Hershey (9) found the vascular system well developed in these internodes at the close of the formative period. Approximately 90 per cent of the bundles present in these internodes in

mature plants were present at the close of the formative period. The first and second internodes above the soil roots were selected for the study of the progress of vascular development in the stem axis for three reasons: They have the maximum diameters of the stem; are more advanced in the development of structures than other nodes above the roots; and through them must pass nearly all the water and minerals used by the plant.

As table 1 shows, the formation of vascular bundles in the first and second internodes above the soil roots progressed rapidly during and immediately following the formative period. When the plants were 37 days of age, the vascular bundles present in these lower internodes were approximately 90 per cent of those present when the development of these internodes was complete.

Although most of the vascular bundles in the lower internodes do the larger part of their developing in the second period of the plant's growth, their number is very largely determined in the formative period. This early establishment of the number of the vascular bundles of the maize plant has considerable significance when the close positive correlation of the number of bundles and diameters of the lower internodes is considered, for it means that both the size and the conductive capacity of the stem axis are practically determined during the formative period and that the factors which have a determinative effect on the number of bundles and the size of the stem operate early in the development of the plant.

TABLE 1. *The number of vascular bundles in the first and second internodes above the soil roots at various intervals in days after planting, calculated in percentages of the number of vascular bundles present in the corresponding internodes of mature plants*

Variety	Percentage of bundles present at the different ages after planting (Age in days)*										
	First internode						Second internode				
	15 days	30 days	37 days	45 days	55 days	110 days	30 days	37 days	45 days	55 days	100 days
Hybrid (274x276)	32	52	79	87	89	100	52	83	86	88	100
Stein's Reid	37	61	99	95	100	100	58	85	89	96	100
McCulloch's Reid	35	68	92	98	97	100	70	85	92	91	100
Osterland's Reid	35	64	86	95	86	100	61	72	90	90	100
Golden King	42	64	89	84	75	100	66	79	85	76	100
Average	36	62	89	92	89	100	61	82	88	88	100

* The average number of vascular bundles present in the internodes above the soil roots in mature stems was considered to be on hundred per cent and was used as a basis for computing the percentage of bundles present at the various dates after planting.

DEVELOPMENT AND NATURE OF THE PERMANENT ROOT SYSTEM

As previously stated the embryonic axis below the node bearing the coleoptile usually functions only during germination. The "temporary" root system is soon supplemented or replaced by the "permanent" roots. (Pl. I, fig. 3). The permanent roots are outgrowths from the regions of the lower nodes of the stem axis. They are stem structures and this

close morphological relationship between the permanent roots and the stem axis is reflected in the interaction of the development of the one upon that of the other.

The first whorl of permanent roots develops from the region of the coleoptile node. The roots of this whorl are relatively small and limited in both conductive and absorptive capacity. Other whorls of larger roots are formed in succession from the nodal regions above until there are generally five to seven whorls of permanent soil roots present in the varieties included in the investigations. Usually five whorls of permanent roots were present at the end of the formative period (Pl. I, fig. 4). The formation of brace roots occurred generally about the time of tasseling and thus after the conductive capacity of the stem axis was completely established.

It is to be noted in Plate I, figure 5 that, in general, the roots of each successive whorl are more numerous and considerably larger than those of the whorls below. Corresponding to the greater number and size of roots in the successive whorls, there is a noticeable difference in the size of the respective nodes from which the successive whorls of roots arise, so that the basal end of the maize stem forms an inverted cone. It is obvious that the number and size of roots in a whorl is correlated positively with the size of the node from which the whorl of roots arises. The development of larger roots and greater leaf area enables the plants to develop larger nodes which in turn can develop larger roots. Thus, any influence that affects the development of stem affects the development of the roots and vice versa.

The development of more and larger roots as the growth of the plant proceeds is the method by which the maize plant and grasses in general increase the absorptive and conductive capacity of their root systems and thus compensate for the lack of a cambium which in dicotyledonous plants is the means of increasing the root system by the unrestricted expansion of the vascular tissues already present.

A cross-sectional view of a maize root, as presented in Plate I, figure 6, shows a row of large metaxylem tracheal vessels encircling the pith. These large tracheal vessels constitute 75 per cent or more of the conductive area of the xylem. Farther from the pith and near the pericycle are the groups of phloem tissues and the smaller tracheal vessels of the xylem. Surrounding the vascular region of the root is a thick cortex of which the outer portion becomes lignified not far back of the absorptive region and thus restricts the further increase in the diameter of the root. This process may account in part for the relative uniformity in the diameter of maize roots throughout their length. The permanent roots of maize emerge from the stem nearly mature in size and when only a few centimeters in length they have their tracheal vessels and other conductive tissue established with no provision for subsequent enlargement. In following the development of a maize root to its origin, one finds that the size and the conductive capacity of the root is largely determined in the "Keimring" of the stem axis in which the root originates. The size of the roots formed varies quite consistently with the diameter of the "Keimring." It follows then that the roots of the five whorls, which generally constituted the root system at the end of the formative period in the varieties investigated, had their conductive capacity and to a large extent

their absorptive capactiy fixed and any stunting effects that had befallen them were beyond remedy. This situation has much significance in view of the fact that the plant depends entirely upon these five whorls of roots when the aerial structures are being determined and also to a large extent throughout its entire life.

The status of the morphological development of the maize plant at the end of the formative period (usually 30 to 35 days after planting), as illustrated by the types of maize included in this investigation, may be summarized as follows: All the nodes, internodes, leaves, and axillary buds the plant will ever have are determined and present in a state of partial development; the tip meristem of the stem axis has been transformed into the primordium of the tassel; approximately 90 per cent of the vascular bundles present in the lower nodes of the stem at maturity are present at the end of the formative period; and, commonly, the root system consists of five or more whorls of roots which have their size and conductive capacity fixed.

RELATION OF THE NUMBER OF VASCULAR BUNDLES IN THE STEM TO THE SIZE OF THE STEM; OF THE NUMBER AND SIZE OF ROOTS TO THE SIZE OF THE STEM; AND OF THE SIZE OF THE ROOTS TO THE CROSS-SECTIONAL AREA OF THEIR TRACHEAE

RELATION OF THE NUMBER OF VASCULAR BUNDLES IN THE STEM TO THE SIZE OF THE STEM

The discovery that the number of vascular bundles in the maize stems investigated was very largely determined during the formative period was followed by an investigation of the relationship of the number of bundles to the size of the stem; for, if the number of vascular bundles in the stem and the size of the stem are closely correlated, then it follows that the size of the stem, too, is very largely determined during the formative period. The conclusions in regard to the relation of the number of bundles to the size of the stem were based upon the number of vascular bundles in the cross-sectional areas of the first and second internode above the soil roots. These internodes were chosen because, as previously stated, they are advanced in development and have the maximum size and conductive capacity of the stem axis.

A significant correlation between the number of vascular bundles and the size of the stem is shown in tables 2 and 3. The coefficients of correlation ranged from .66 to .69 and from .67 to .72, according to the diameter considered. It follows, therefore, that the size of the stem was largely determined during the formative period as well as the number of vascular bundles in the stem.

The close correlation between number of bundles in the first and second internodes above the soil roots, as shown by the coefficient .74, in part justifies the assumption that the anatomical status of the largest node, which is nearly always the first or second internode above the soil roots, may be taken as representative of the entire stem.

Furthermore, as shown in table 4, the size of the vascular bundles varied with their number. In the small stems the vascular bundles were about 36 per cent fewer in number and 27 to 30 per cent less in size than those of the larger stems. This means that a maize plant with an undersized stem suffers two reductions in its conductive capacity; namely,

TABLE 2. *The variation in number of vascular bundles in the first and second inter-nodes of a commercial variety (McCulloch Yellow Dent)*

	First internode			Second internode		
	Diameters in mm.		Number of vascular bundles	Diameters in mm.		Number of vascular bundles
Number	Large	Small		Large	Small	
1	20	17	500	24	20	540
2	21	20	520	22	21	560
3	20	18	560	23	19	640
4	19	18	560	24	21	640
5	21	20	560	27	23	660
6	17	16	600	20	17	720
7	23	21	640	23	20	640
8	24	24	660	28	26	720
9	28	23	700	32	26	800
10	24	23	700	26	24	740
11	24	22	720	27	24	760
12	30	24	720	34	28	740
13	26	23	740	30	26	880
14	32	30	740	35	31	880
15	28	26	760	33	29	880
16	27	25	760	30	26	800
17	26	24	820	29	25	800
18	28	25	880	28	24	860
19	30	27	880	32	27	840
20	25	22	840	30	25	880

TABLE 3. *Coefficients of correlation among various characters and age of the maize stem*

Variable	Age in days	Height of stem	Large diameter	Small diameter	No. of vascular bundles in first internode	No. of vascular bundles in second internode
	A	B	C	D	E	X
A. Age in days		.96	.73	.71	.43	.46
B. Height in stem			.77	.74	.39	.44
C. Large diemeter				.98	.69	.66
D. Small diameter					.72	.67
E. Number of vascular bundles in first internode						.74

in its number and its size of vascular bundles, both of which are largely determined during the formative period of the plant's growth.

The data in table 4 pertain to plants grown in the greenhouse, of which all were considerably below normal in size. It is probable that a comparison of large and small plants grown in the field would show a more marked difference. The observations were made on the largest internode, usually the first internode above the soil roots, of 30 approxi-

TABLE 4. *Relative number and size of vascular bundles in large and small maize stems (based on studies of the largest internode)*

Type of stem	Cross sec. area	No. in stem	No. per sq. cm. of stem	Size in rind	Size in pith	Distance between vascular bundles Rind	Pith
	sq.cm.			microns	microns	microns	microns
Large stems	.54	309	572	195x145	207x248	101	456
Small stems	.17	198	1165	140x105	104x165	70	321

Note: All results are given as averages.

TABLE 5. *Data showing the relation of the number of roots in the upper whorl of the soil roots and of the cross-sectional area of the roots of each whorl of soil roots to the cross-sectional area of the stems*

Cross sectional area of stems sq. mm.	No. of roots at upper node	1st node	2nd node	3rd node	4th node	5th node	6th node	7th node	8th node
804.24	26	23.75	21.645	23.75	15.890	9.621	1.767	1.225	
731.99	19	16.687	14.32	15.896	9.621	12.566	0.7854	0.7854	
706.96	16	28.2744	25.698	23.75	9.621	5.937	1.225		
706.86	23	25.698	28.274	25.698	16.687	15.904	9.621	2.405	
637.744	20	19.635	21.645	12.5664	4.908	2.405	1.225		
615.75	23	21.645	28.274	33.174	12.566	5.937	7.068	1.225	
595.74	17	19.635	35.814	24.698	12.566	5.937	0.7854	0.7854	
572.55	18	21.645	28.274	21.645	7.068	7.854	0.7854		
530.93	21	16.687	12.566	15.904	15.904	9.621	1.225		
530.93	20	31.196	15.904	16.587	21.645	7.068	0.7854	0.7854	
520.93	18	17.718	23.750	9.621	12.566	4.908	1.225		
508.93	20	21.645	16.687	9.621	5.937	4.908	4.908	0.7854	
452.39	15	25.698	23.750	25.698	15.896	9.621	4.908	0.7854	
452.39	16	21.645	19.635	7.068	4.908	3.1416	1.225		
433.54	20	15.904	19.635	9.621	8.293	7.068	2.405	0.7854	.7854
433.54	22	12.5664	15.896	11.045	4.908	2.405	0.7854	0.7854	
329.86	13	15.904	11.042	12.664	7.068	4.908	2.405	0.7854	.7854
298.452	17	9.621	15.896	11.045	7.068	0.7854	0.7854		
283.52	16	9.621	9.621	7.068	5.937	3.1416	1.225		
240.52	16	9.621	12.566	4.908	7.068	4.908	7.767		
240.52	16	9.621	12.566	4.908	7.068	4.908	7.767	--	
213.628	15	7.068	9.621	5.937	2.405	0.7854	0.7854		
176.715	14	3.1416	4.908	0.7854					
164.934	9	9.621	11.042	4.908	9.621	0.7854	0.7854		
153.938	10	9.621	7.0686	4.908	1.767	1.225	0.7854		
148.40	19	8.293	11.042	11.042	11.042	5.937	2.405	0.7854	
132.73	8	4.908	4.908	2.405	1.225	0.7854			
113.00	6	11.042	4.908	2.405	1.225	0.7854			
95.033	6	1.767	4.908	4.908	4.908	1.767			
70.686	8	4.908	3.1416	4.908	1.225	0.7854			

mately mature plants, so selected as to include 15 of the largest and 15 of the smallest individuals.

A comparison of the number of bundles per square centimeter of cross sectional area shows that the bundles are much more crowded in the small stems notwithstanding their reduction in number. This same feature is shown again in the last two columns of the table where distances between bundles are compared.

RELATION OF THE NUMBER AND THE SIZE OF ROOTS TO THE SIZE OF THE STEM

The relation of the number and size of roots to the size of the stem was determined from measurements of 100 field plants of a common strain of Reid's Yellow Dent. The plants were grown singly and two to three feet apart so as to avoid to a large extent the effects of root competition. The size of the stems refers to the cross-sectional area of the internode just above the soil roots. The relation of the number of roots to the size of the stem was determined by comparing the number of roots in the uppermost whorl of the soil roots and the size of the largest internode. The average cross-sectional area of the roots at each node was determined from measurements of six roots at each node or of all the roots at the nodes which had six or less roots. Table 5 presents a representative sample of the data obtained.

Although there are some rather marked inconsistencies shown in table 5, in general, there is a significant correlation between the features under consideration. Between the size of the stem and the number of roots in the uppermost whorl of soil roots and between the size of the stem and the average cross-sectional area of the roots at the five upper nodes, the correlation coefficients, shown in table 6, are highly significant. Between the size of the stem and the average cross-sectional area of the roots at the lowest nodes, there was much less correlation. The probable explanation is that the lowermost whorls of roots are formed while the plant is dependent upon the endosperm and consequently less influenced by environmental factors. Furthermore, the lowermost roots are all small and consequently less subject to marked variations in size.

THE RELATION OF THE SIZE OF MAIZE ROOTS TO THE NUMBER AND TOTAL CROSS-SECTIONAL AREA OF THEIR TRACHEAL VESSELS

The tendency of the number and size of maize roots to vary with the size of the stem is especially significant in its bearing upon the absorptive and conductive capacity of the maize root system. An attempt was made to determine quantitatively the relation of the size of maize roots to their conductive capacity by comparing the average cross-sectional area of the roots with the total cross-sectional area of their tracheal vessels. The determinations were based upon the measurements of 150 roots from 50 plants of a common variety of Reid's Yellow Dent. Most of the plants were taken from the field. A few were grown in the greenhouse.

As previously stated and shown in figure 6, the tracheal vessels of maize roots consist of two types, the large metaxylem tracheae that border the pith and the smaller but more numerous tracheal vessels that are located more nearly in the region of the phloem and pericycle. A comparison of the cross-sections of large and small roots as shown in figure 7 disclosed very little variation in the size of either of the types of vessels in the roots of the variety investigated. Whether or not different varieties

TABLE 6. *Correlation coefficients between the size of maize stems and the number of roots in the uppermost whorl of soil roots, and between the size of maize stems and the average cross-sectional area of the roots at each node. Based on data shown in table 5*

Between size of stem and number roots in uppermost whorl of soil roots	Between the size of the stem and the average cross-sectional area of the roots at each node						
	1st node (counting downward)	2nd node	3rd node	4th node	5th node	6th node	7th node
.80	.82	.81	.79	.65	.735	.2864	.4679

of maize differ in the size of their tracheae is still to be determined. The variations in the total cross-sectional area of the tracheae of the maize roots is attributable, therefore, mainly to the variation in the number of the vessels.

The data in table 7 show that the number of tracheae and also the total area of the tracheae in maize roots vary quite consistently with the size of the roots. The correlation coefficients, given in table 8, which are highly significant, show more convincingly the close relationship of the features under consideration.

The data in table 7 show further that the large metaxylem vessels of maize roots afford the greater part of the conductive capacity if cross-sectional areas can be taken as measures of conductive capacity. The cross-sectional area of the large tubes was quite commonly more than 80 per cent of the total cross-sectional area of the root tracheae.

SUMMARY AND DISCUSSION

The morphological development of several varieties of maize plants are described with particular reference to the differentiation of stem structures and the relation of the following: The size of the stem to the number of its vascular bundles and to the number and size of its permanent roots; and the average size of the roots to the number and total cross-sectional area of their tracheal vessels.

It was found that almost all the structures of the plant were formed during the first 30 to 40 days following planting; namely, all the nodes, internodes, leaves and axillary buds, also the tassel, at least five whorls of permanent roots, and 90 per cent of the vascular bundles in the lower internodes of the stem. In most of the maize plants observed, all the structures listed above were differentiated prior to the thirty-fifth day after planting. The period of differentiation was followed by a period of 35 to 40 days in which almost all the structures differentiated during the previous or formative period developed to mature size. This period of development was followed by pollination, fertilization, and the maturing of the kernels.

Of the three rather distinct periods in the life cycle of the maize plants, the first or formative period much surpassed the others in determinative effects on the final achievement of the plants, because during this period nearly all the structures upon which the future development

TABLE 7. *Data showing the relation of the cross-sectional area of maize roots to the number and to the total cross-sectional area of both their large and small tracheae*

Cross section area of roots sq. mm.	Number of large tubes	Number of small tubes	Cross section area of large tubes	Cross section area of small tubes	Area of large tubes in percentage of area of roots	Area of small tubes in percentage of area of roots
28.2744	80	240	.6283	.2094	.0222	.007
21.9912	60	180	.8369	.1561	.038	.0071
21.9912	65	195	.6942	.1702	.031	.0077
21.9912	60	180	.8369	.1561	.038	.0071
21.9912	65	195	.6942	.1702	.031	.0077
21.9912	80	240	.4348	.2094	.029	.0095
19.635	60	180	.3261	.0427	.026	.0021
19.635	60	180	.6059	.1561	.032	.0079
19.635	80	240	.6283	.2094	.032	.0106
19.635	75	225	.8011	.1964	.0408	.01
19.635	75	225	.5891	.1964	.03	.01
15.904	55	165	.2989	.0360	.019	.0022
15.904	40	120	.5379	.1047	.033	.0065
15.904	50	150	.5341	.1308	.033	.0082
15.904	36	108	.5020	.0942	.031	.0059
12.566	30	90	.3204	.0785	.025	.0062
12.566	60	80	.4712	.0698	.038	.0055
12.566	45	135	.4806	.0295	.038	.0023
9.621	40	120	.3142	.1047	.0326	.0109
9.621	45	135	.3534	.1178	.0364	.0122
9.621	48	144	.2608	.1257	.027	.013
9.621	28	84	.2990	.0733	.3079	.0076
7.068	30	90	.1630	.0196	.023	.0027
7.068	28	84	.2199	.0733	.0308	.0103
7.068	40	120	.2074	.1047	.0304	.0148
7.068	18	54	.1923	.0471	.027	.0066
4.908	18	54	.1923	.0471	.039	.0096
4.908	30	90	.2356	.0785	.048	.016
4.908	40	120	.3142	.1047	.0631	.0213
4.908	24	72	.1004	.0628	.0263	.0128
3.1416	12	36	.1282	.0314	.0409	.01
3.1416	12	36	.1272	.0314	.0409	.01
3.1416	36	108	.1953	.0942	.0621	.03
3.1416	20	60	.1087	.0131	.034	.0042
1.7671	20	60	.1087	.0524	.0615	.0296
1.7671	12	36	.0652	.0079	.0369	.0044
1.7671	14	42	.0761	.0367	.043	.0207
0.7854	11	33	.1176	.0287	.149	.0366
0.7854	10	30	.0785	.0262	.10	.0333
0.7854	14	42	.0761	.0092	.094	.0116
0.7854	18	54	.0987	.0118	.124	.0142
0.7854	16	48	.0870	.0105	.11	.0133

of the plants depended were established and to a large extent fixed in both number and size.

During this period the number of nodes, internodes, leaves and axillary buds were determined by the transformation of the tip meristem

TABLE 8. *The coefficients of correlation between the cross-sectional area of maize roots and the number and the total cross-sectional area of their large and small tracheae*

Between number of large tubes and cross-sectional area of roots	Between number of small tubes and cross-sectional area of roots	Between the cross-sectional area of roots and total cross-sectional area of large tubes	Between the cross-sectional area of roots and total cross-sectional area of small tubes
.9175	.9146	.9121	.8162

into the tassel primordium. Ninety per cent of the vascular bundles in the first and second internodes above the soil roots and at least five of the seven whorls of roots generally present at maturity were formed during the formative period. The positive correlation of the size of the lower internodes and the number of their vascular bundles showed that the size of the stems, too, was largely determined during the formative period. The close positive correlation of the size of the stem and the number and size of the permanent roots and of the size of the roots and their tracheal vessels leads to the conclusion that all these features were largely determined during the first 30 to 40 days and generally before the thirty-fifth day after planting.

The early determination of structures is especially significant in maize in view of the fact that there is very little provision whereby structures can enlarge after they are established. The anatomy of the maize plant makes no provision in the roots and very little in the stems for the recovery from stunting effects that occur during the establishment of these structures. Favorable environment and the best cultural methods during the developmental and following period are necessary to bring about the maximum efficiency in the functioning of the structures present, but they cannot eliminate or mitigate to any great extent the structural deficiencies imposed upon the plant during the formative period. On the other hand, it is well recognized that unfavorable conditions and cultural methods during the later periods can render ineffective the advantages of a perfect structural organization developed during a favorable formative period. A view of the situation which takes into account the fact that nearly all of the structural features of both stem and roots which have to do with the mechanical support of the plant and with the supplying of the water and minerals to the aerial portions are established and largely fixed numerically and quantitatively during the formative period leads to the conclusion that the maximum possible harvest of a given maize crop is very largely determined during the formative period.

From the foregoing conclusions it follows that influences which have stunting effects on maize plants tend to be most serious when they operate early. Such environmental factors as competition and shading, for example, produce the most injurious effects during the formative period.

Some interesting questions arise here regarding the interactions of roots, stem axis, and leaves upon each other. The close correlation between the size of the maize stem and the number of roots in the upper whorl, and size of roots in the different whorls shows that a reduction in the growth of one was followed by a reduction in the subsequent growth of

the other. One would naturally expect a similar close relationship to exist between the development of the stem axis and the leaves. It follows that any influence affecting the development of one of these structures similarly affects the development of the others. Eisele (7) found that the stems of maize plants grown five in a hill were less than half the size of the stems of plants grown singly. In view of the close positive correlation between size of stem and number, size, and conductive capacity of roots, as shown by the data obtained by the authors, there must have been a corresponding reduction in the root systems. He found, however, that the reduction in the size of the stem was pronounced before any reduction in leaf area was recognizable. In other words, the effect was first manifested either in the roots or the stem and the effect in the leaf area was secondary. Hershey (9) obtained a marked reduction in the size of maize stems when the plants were grown under a cheese cloth shade. Here again, in view of the close positive correlation between size of stem and the number and size of roots, there must have been a similar reduction in the size of the root systems.

In the pruning of fruit trees Chandler (5) found that with a reduction in leaf surface and consequently in the supply of photosynthetic foods, root growth was often checked much more than top growth. Apparently the structures nearest the place where the food is made have first claim on the food supply. In the maize plants grown under cheese cloth and possibly in those grown crowded in the hill, it is probable that root growth was checked more than the growth of the aerial parts.

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DESCRIPTION OF PLATE I

Fig. 1. Embryo of maize (a variety of Reid's Yellow Dent) at time of planting, showing the upper end of the embryonic axis with the apical meristem (M) and coleoptile node (C) indicated. The coleoptile node and portions of the embryonic axis above are the only portions of the embryo present in the mature plant.

Fig. 2. Lengthwise section through the apex of a maize stem 37 days after planting. The apical meristem has been transformed into a tassel primordium (t) and several axillary buds (b) (potential ears) are recognizable in the leaf axils.

Fig. 3. Seedling of maize showing the lowermost or coleoptile node (c) of the maize stem from which the first permanent roots arise. All the seedling below this node usually ceases to function soon after germination.

Fig. 4. Plant 45 days of age from which leaves have been removed to expose to view the tassel at the apex and the axillary buds at the nodes. This plant was in the early stage of rapid stem-elongation. Note that the stem base is an inverted cone.

Fig. 5. Maize plant near tasseling stage showing the usual difference in size of nodes bearing roots, in size of the roots of the different whorls, and the uniformity in size the roots maintain throughout their length.

Fig. 6. Cross section of a maize root showing the large metaxylem vessels (l) and the small xylem vessels (s).

Fig. 7. Cross sections of a large and a small maize root showing the uniformity in size of tracheae regardless of the size of roots.

PLATE I.

SOME NEW ASPECTS OF MAIZE SMUT[1], [2]

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Corn smut produces galls on all aerial parts of the corn plant but the most common place of attack is the node. It has been observed that nodal and ear infections are prevalent in some years and rare in others, and that these infections vary considerably in abundance in neighboring fields and in different sections of the same field. Furthermore, it has been observed that these galls appear late in the development of the corn plant.

The opinion is rather general that smut galls result from infection of the axillary bud at the point where the smut gall originates. However, results of our study as set forth in the following presentation have led to the belief that the corn smut organism infects the plant at an early stage of development and that subsequent parasitism and expression of the smut are dependent upon the development of its host plant.

Artificial inoculations with corn smut at the nodes never have given results typical of those found in the field. The hypothesis in these studies was that infection took place early in the development of the corn plant by way of the spiral whorl. In securing infections in this way, it was found that both the age of the plant, and surface tension of the inoculating fluid were important factors.

A sporidial suspension in carrot decoction with a surface tension of 47.0 dynes per sq. cm. gave an average of 30 per cent of plants with infected leaves but no smut galls were produced. A spore suspension in 0.5 per cent acetone-carrot decoction with a surface tension of 43.6 dynes gave 38.4 per cent infection with galls produced on 7.6 per cent of the inoculated plants. A spore suspension in 1.0 per cent acetone-carrot decoction gave 53.0 per cent infection with galls on 30.7 per cent of the inoculated plants. The 0.5 per cent fish oil soap-carrot decoction inoculum resulted in 77.0 per cent infection with galls on 38.4 per cent of the inoculated plants, while the spore suspension in 1.0 per cent fish oil soap-carrot decoction which had the lowest surface tension of all the solutions used, namely 34.0 dynes, gave 92.3 per cent infection with galls produced on 69.2 per cent of the inoculated plants.

As a result of the foregoing experiment the spore suspension in 1.0 per cent fish oil soap-carrot decoction was selected as the inoculum for further study. A total of 500 sweet corn plants, variety Golden Bantam, were inoculated when about one foot tall. Of these 92.0 per cent became infected and on 32.0 per cent of the plants smut galls were produced. However, 54.4 per cent of the smut galls produced did not make their appearance until late in the development of the plants. In an experiment in which 500 similar plants were inoculated with a sporidial sus-

[2] Abstract.

pension in carrot decoction, 36.6 per cent of the plants became infected. When 150 plants reputed as very resistant and showing an average of only 1.9 per cent natural infection in the field were inoculated with a sporidial suspension in a 1.0 per cent fish oil soap-carrot decoction solution galls were produced on 30.0 per cent of the plants.

The leaf sheaths were stripped from 1985 plants which were exposed to natural and artificial infection during the summer of 1934. Many small smut galls aggregating 39.3 per cent of the total infection, which had not grown sufficiently large to rupture the leaf sheaths were found at the nodes, indicating the need to remove the leaf sheath when varieties are tested for resistance to obtain a more nearly complete picture of total infection.

Since nodal infections usually appear late in the season and often in times of extensive drouth it appeared that the smut mycelium may have been dormant in the nodal buds for a long period. A series of field and laboratory experiments was conducted during 1931 to 1934 inclusive to determine whether this might be the case. Field corn variety Reed's Yellow Dent was planted on or near May 10 of each year and inoculated when about one foot tall by dropping one and one-half to two c.c. of a sporidial suspension in a 1.0 per cent fish oil soap-carrot decoction solution into a spiral whorl of each plant. An equal number of non-inoculated plants were held as checks. About the middle of August of each year smut readings were taken on all plants with special attention to nodal infection. Immediately thereafter the plants were injured to increase axillary bud development and to determine the effect of such increased bud development on the production of nodal smut galls. A series of inoculated and non-inoculated plants was treated in the following ways, (1) tops removed about the ear, (2) ears removed, and (3) tops and ears removed. Uninjured plants were held as checks and final smut readings were taken near the last of September.

Injury to the corn plants by any of the methods mentioned did not result in a very large increase in the number of infected plants. However, it did result in a large increase in the number of nodal smut galls over the number obtained in the uninjured check plants. The results obtained by injury to the inoculated and non-inoculated plants were of the same nature, except that there was a higher percentage of infected plants and a greater number of smut galls in the inoculated plants than in the non-inoculated. Results of four years' experiments figured on a per-plant basis showed that the removal of the tops of the non-inoculated plants did not give a significant increase in the number of nodal smut galls. However, removal of the ears gave an increase of 23.3 per cent over the checks and removal of the tops and ears gave an increase of 45.1 per cent over the checks. In the case of the inoculated plants removal of the tops gave an increase of 20.4 per cent in the number of nodal smut galls over the number obtained in the inoculated uninjured check plants. Removal of the ears gave an increase of 15.6 per cent over the checks and removal of tops and ears gave an increase over the checks of 31.7 per cent. Similar results were obtained by inhibiting pollination by covering the silks with a paper bag. On a per-plant basis 30.2 per cent more smut galls were produced on the inoculated plants than on those exposed to natural infection.

The results obtained in the foregoing experiments indicating the presence of unexpressed infection suggested the examination of axillary buds for the detection of smut mycelium in order to determine whether more buds were actually infected than was indicated by the number of smut galls produced. A series of sweet corn plants, variety Golden Bantam, was planted in the greenhouse and inoculated when about 30 days old. At 14 days after inoculation, and at intervals thereafter for 67 days, 10 plants at a time were selected at random and all the axillary buds on these plants were examined for the presence of mycelium. Examination was made by cutting longitudinal free hand sections from each bud. The sections were stained with cotton blue in lactophenol and counterstained with eosin, staining the mycelium a dark blue and the plant tissue a light pink color. Histological sections and prepared slides were made to verify the free hand examinations. A total of 262 axillary buds from 50 plants were examined and of this number 140, or 50.7 per cent, were found to be infected with smut mycelium. This number, or 140 infected axillary buds, is greater than the number of smut galls produced on 50 plants, therefore a large percentage of the infected buds may never produce smut galls and the presence of the mycelium in them may never be expressed. Such findings show that a corn plant may be susceptible to smut infection and may even be infected but due to failure of the axillary buds to develop may not express the infection.

The foregoing results may lead to the explanation of the prevalence of corn smut in dry years. If, as indicated, nodal infections occur only when the plants are young and become evident only when the dormant buds become active, then nodal smut boils are dependent on factors which tend to stimulate activity in the axillary buds of the corn plant. This stimulation is very evident in dry years when there are more barren and poorly pollinated ears resulting in excess food and bud stimulation.

THE TRANSLOCATION OF CARBOHYDRATES IN MAIZE[1]

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Maize ranks among the important agricultural crops of the world and is one of the most efficient plants of the temperate zones in the production of dry matter. The economic importance of the plant justifies more careful studies of its physiology than have been published to date, while its relatively high synthetic efficiency suggests that a careful study of the production and utilization of carbohydrates by maize might contribute to our knowledge of limiting factors in photosynthesis.

The present paper is particularly concerned wtih translocation in the maize plant as affected by photosynthesis, sugar gradients and other factors, and as affecting rates of photosynthesis and storage within the plant.

EXPERIMENTAL

THE RELATION OF FRUITING TO TRANSLOCATION IN MAIZE

A number of preliminary experiments were run to determine the relation of leaf area and other factors to grain yield; the yield of grain being considered a partial measure of total translocation. In the 1929 experiments from which most of the data shown are taken, treatments were replicated six times with single row, 20-hill plots. In the 1927 and 1928 experiments four replications were used with the same size plots. The plots were arranged to throw similar treatments together; when this was not possible guard rows were inserted. For example, if row one were normal and row two, lower 75 per cent of leaves left, row three would be lower 50 per cent of leaves, etc. Row two thus had as much advantage over row three as it had disadvantage in comparison with row one. This arrangement resulted in the partially defoliated plots receiving maximum sunlight and minimum root competition. As a check upon border effect, a row with the upper leaves removed (lower 25 per cent left) was placed between normal rows where it received maximum competition. In the early experiments the exposed row in the regular series, which had the lower 25 per cent of its leaves remaining, yielded 1.2, and in the late experiments 2.0 bushels an acre more than the same treatment with overshadowing, normal guards. Neither difference was statistically significant. These results indicate that border effect was not a serious factor in the experiments.

The data obtained in these defoliation experiments were very clear cut in showing the dependence of grain yield upon leaf area. Additional data supporting this conclusion have been obtained by Dungan (3) at Illinois and by Eldredge (4) at this station. The relationship between leaf area and yield is shown in figure 1 and table 1. Removing all of the leaves at tasseling stopped the development of the ear shoots and no grain was produced.

TABLE 1. *Relation of leaf area to yield of maize, 1929*

Leaves left after defoliation	Yield in bushels per acre	
	Defoliated at tasseling	Defoliated at glaze
None	0.0	33.8 ± 0.5
Lower 25 per cent	5.3 ± 0.5	46.2 ± 1.2
Upper 25 per cent	19.8 ± 1.1	43.4 ± 0.4
Lower 50 per cent	36.0 ± 0.8	49.9 ± 2.1
Upper 50 per cent	43.8 ± 1.9	53.3 ± 2.7
Lower 75 per cent	56.0 ± 1.6	58.9 ± 1.2
Upper 75 per cent	56.9 ± 2.2	61.7 ± 1.7
Check—all leaves	61.2 ± 3.8	65.8 ± 1.8

When 25 per cent of the leaves were left on the tasseling plants the yield was 300 per cent greater when the leaves were at the top of the plant. The upper 50 per cent of the leaves also outyielded significantly the lower 50 per cent of leaves (table 1). These differences cannot be ascribed to leaf efficiency for in the late treatments there were no significant differences in the yields due to age or position of leaves on the stalk (fig. 2).

The plants with the lower 25 per cent of their leaves remaining and producing 5.3 bushels of dry shelled grain an acre, formed only a few scattered grains on each cob. These grains were normal in size and 23 per cent larger than the grains produced by plants with the leaves at the top. Most of the ovules on the plants with the leaves left below the ear shoot at tasseling failed to develop, and total translocation to the ear was low. When the same leaf area was left above the ear more than four times as many ovules developed.

Experiments with woody plants (9), in which translocation can be checked conveniently by cutting away the phloem, have shown that the normal path of elaborated food is downward from the leaves. Harvey and Murneek (6) have reported that defoliation of a single spur without disturbing the adjoining leaves, prevents the formation of fruit buds and flowers in apple. On the other hand, Haller (5) finds that normal fruits may be produced on defoliated apple limbs when the nearest leaves are as much as ten feet from the fruit, provided defoliation is delayed until after the

Percentage of leaves remaining.
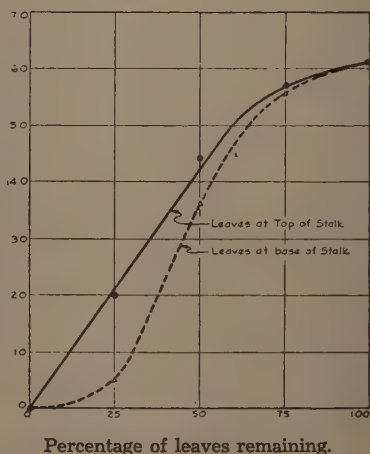
Fig. 1. The relation of the percentage and location of leaves left on silking maize plants to the yield of grain.

In plants stripped at this stage the leaves located below the ear shank were significantly less effective than those above.

fruit is set. Dungan (3) reports that the leaves of maize suckers supply food materials to the ear when the main stalk is defoliated in the milk stage, but not when it is defoliated before fertilization.

The failure of suckers and lower leaves to supply enough food for ovule development suggests that the elaborated food materials of the young corn plant move downward, as they have been shown to do in trees, and only those plants with leaves at or above the ear shank moved enough food to the young ear to insure the development of a considerable number of ovules. After fertilization, translocation conditions within the corn plant are apparently changed as they are in apple, and the developing fruits dominate the translocation processes of the plant. The failure of the development of the ovules and silks, or possibly of the fertilized egg, is emphasized in this report because of the abundant pollen available from nearby plants. The tassel of defoliated plants was affected, but since the tassel was developed further than the ear at the time of the early defoliation treatments, and there was no possibility of a pollen shortage, no study of anther and pollen development was made.

Percentage of leaves remaining.

Fig. 2. The effectiveness of upper and lower leaves of maize plants with fertilized and developing ears.

After fertilization the lower leaves of the maize plant were as effective, area for area, as the upper leaves.

Normally, half of the leaf area of the maize plant is above the ear and half below. When the leaves were removed at tasseling the upper leaves were significantly more efficient in grain production than the lower until 75 per cent of the area was left (fig. 1). With these treatments at least three large leaves were left above the ear. Removing the upper leaves exposed the lower leaves to adequate light, and the greater age of the lower leaves did not prevent their showing an efficiency as great as that of the upper leaves in the late experiments (fig. 2). The data would seem to be best explained by assuming, as we have, that upward translocation from the lower leaves to the ear is not initiated until after fertilization and the beginning of embryo development.

Additional evidence on the dominant effect of the fertilized ear upon translocation in maize was obtained by noting the relation of leaf area and developing ovules to the root system of the plant. Plants developing normally made comparatively little root growth after fertilization. Plants on which ear formation was prevented showed an abnormally vigorous root system up to the time the plants were killed by frost. On the other

TABLE 2. *The relation between the ratio of leaf area to grain and the late season vigor of maize*

Treatment	Yield in bu. acre	Ave. wt. kernels in gms.	Percentage of stalks dying prematurely
None	61.2 ± 3.8	0.318	8
¾ of leaves removed before fertilization	5.3 ± 0.5	0.303	0
¾ of leaves removed after fertilization*	46.2 ± 1.2	0.244	73

* Plants harvested at the date of this late defoliation produced 33.8 bu. of chaffy grain. The remaining 25 per cent of the leaf area thus produced 12.4 bu. of grain in approximately half of the time required to produce 5.3 bu. with the corresponding early defoliation.

hand, when one-half to three-fourths of the leaves of maize plants were removed soon after fertilization, the weakening of the roots was so pronounced as to have been apparently a major factor in the early death of the stalks, with the consequent production of chaffy grain. The data are presented in table 2. When the majority of the leaves were removed before fertilization the ovules did not develop and the food supply for the stalk and roots was normal or better than normal. When one-half to three-fourths of the leaves were removed at the time that the grain crop was about one-half developed, the large number of developing fruits in relation to the sharply reduced leaf area resulted in the premature death of the roots, and in the subsequent death of the tops. A comparison of figures 5 and 6 shows that the bases of the normal fruiting stalks contained less than one-third as much sucrose as did the defruited plants, and removing some of the leaves from the fruiting stalks should have further emphasized this effect of the ear.

The flattening of the upper portions of the curves in figure 1 also may be explained upon a basis of translocation. When the leaf area was reduced to a relatively low value (25 to 75 per cent of normal) the fully developed translocation machinery of the plant would have been expected to effect the rapid removal of food materials from the leaves. With a relatively large leaf area for the same or an only slightly larger number of developing ovules, probably translocation became a limiting factor in grain development. With a delay in the removal of photosynthate from the leaves we might expect a tendency toward the reversal of some of the photosynthetic reactions with a consequent loss in leaf efficiency. The loss in efficiency is shown by the data although direct evidence that the photosynthetic processes were reversed is not available.

Injuries to the leaf and stalk which did not decrease the leaf area had little or no effect upon yield. The data from some of these experiments are given in table 3. The lower internodes of maize stalks were bruised, in one case so badly that 97 per cent of the stalks were down shortly after treating, without significantly reducing the yield. Slitting the leaves lengthwise and leaf mutilations which did not result in a loss of leaf area also failed to affect the yield. Either photosynthesis and translocation were not disturbed by these treatments, or, more probably, the disturbance did not result in the 10 or 15 per cent loss of effective leaf area which might

TABLE 3. *The effects of bruising the stalk or slitting the leaves upon the yield of maize*

Stalks bruised 12 times	Check yield bu. per acre	Yield of treated plants	Loss due to treat-ment	Odds of signifi-cance	Percentage bruised stalks broken
1928 experiments	48.0	44.9	3.1	2.5–1	97.1
July, 1929	61.2	58.9	2.3	1.0–1	4.0
August, 1929	65.8	60.5	5.3	3.4–1	55.0
Leaves slit 4–6 times					
July, 1929	61.2	57.7	3.5	1–1	
August, 1929	65.8	64.2	1.6	1–1	

have been expected from figure 1 to give a significant reduction in yield under the conditons of the experiment.

THE EFFECT OF DEFRUITING UPON THE DRY MATTER OF THE MAIZE PLANT

In the experiments just described the leaf area was varied and variations in storage area were incidental. In the late removal of the leaves the storage capacity of the ear in so far as this is determined by the number of developing ovules, was unaffected. In parallel experiments a study was made of accumulation of dry matter in plants from which all developing ears were removed. Mason and Maskell (12) have shown that sugars move on a concentration gradient in the bark of cotton. Removing the ear should upset any gradients present in the corn plant by eliminating the principal storage organs. The treatment resulted in a sharp reduction in the total dry matter accumulation in the tops of the defruited plants, as may be seen in table 4. The stalks of the defruited plants

TABLE 4. *The effect of removing the ear on the dry weight increment of the maize plant*

Series	Dry. wt.check		Dry wt. defruited		Dry wt. increments after stripping		
	Ears	stalks	Strippings	Stalks	Check	Defruited	Ratio
1928	41.6	38.4	19.4	51.9	23.2	15.5	1.5–1
Early 1929	17.4	10.7	4.12	16.8	14.39	7.21	2.0–1
Late 1929	18.9	13.6	10.6	16.0	11.9	6.0	2.0–1

weighed 35 per cent more at maturity than the stalks of normal plants carrying an ear, but this increase in stalk weight was approximately only one-half as great as the increase in ear weight of the check plants during the same period. The defruited plants had a more vigorous root system at the end of the period and undoubtedly made a greater root growth under the stimulus of higher food levels in the plants (7). Although no measurements of root growth were made, the force required to pull the defruited plants on September 15 was estimated to be three to five times the force required to pull normal plants bearing ears. The defruited plants also developed a deep red anthocyanin pigmentation, and

the stalks appeared to be stiffer although breaking tests showed that the 11 to 23 per cent greater average strength of defruited stalks was so variable in individual measurements as not to be statistically significant with a 20-stalk example.

DIURNAL VARIATIONS IN THE RATE OF TRANSLOCATION IN MAIZE

The conventional picture of translocation assumes that it is characteristically a night time process. Curtis' (2) experiments on the effect of temperature on translocation might suggest that cool nights would be less favorable for translocation than the warmer day. The normally higher sugar content of the leaves in the day should also accelerate translocation at this time. On the other hand, the generally greater growth of plants at night might be expected to reduce sugar concentrations within the plant and so increase movement at night. Loomis (8) has shown, however, that maize makes its most rapid growth in the late afternoon and early evening and morning, or on cloudy days when the air temperature is high, but a water deficit is not maintained within the plant shoot by a high transpiration rate. Diurnal sugar curves for the leaves and stalks of maize in the silking stage are shown in figure 3. Similar curves made at later development stages differ only in showing a very much higher percentage of sucrose in the stem with the same diurnal fluctuation. It is probably impossible to fix with certainty the sugars of photosynthesis and translocation from data of this type. Throughout all of our work, however, sucrose has been the only sugar, and with dextrin, the only carbohydrate, to show appreciable systematic daily changes in concentration in any organ of the vegetative plant. The sucrose curve for the leaves suggests that translocation from these organs reaches a maximum at 4 p. m. and is practically complete by 10 p. m. These hours are also the hours of normally greatest growth (8). A small portion of the rapid drop in sucrose may be attributed to the formation of dextrin within the leaf, but the percentage of dextrin is never large and it normally reaches a maximum at 6 p. m. or before; too early to seriously affect our conclusion that translocation is most active in the afternoon and early evening.
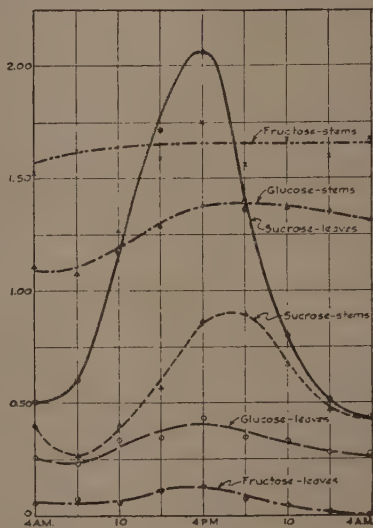
Fig. 3. Diurnal variations in the sugar concentrations of the leaf and stem of silking maize. Sampled July 15-16, 1934.

Sucrose would appear to be the sugar accumulating in the leaves and moving to the stems. At this stage sucrose shows a positive gradient from the leaves to the stems and ear shoot, but after fertilization the sucrose content of stalks and ear shanks rises sharply until all gradients become negative.

TRANSLOCATION GRADIENTS IN THE
MAIZE PLANT

The earlier work of Mason and Maskell (12) suggests that a concentration gradient is a primary factor in the translocation of organic food materials in plants, although unknown factors increase the rate of movement of sugars in the bark of the cotton plant to 50 times the rate which would be expected from diffusion acting alone over the observed gradients. In a later paper Phillis and Mason (12) found that sugar was moved against a concentration gradient from the border parenchyma of the leaf mesophyl into the phloem, but that once within the phloem it moved on a positive gradient to the point of utilization. These results indicate the possibility of moving sugars from a lower to a higher concentration through the action of living cells, whereas the usual concepts of the movement of solutes within plants do not cover this type of "pumping" action. The net conclusions of the Trinidad work on translocation in cotton seem, however, to be that although movement against a gradient or vital "pumping" is possible, most movement of carbohydrate and organic nitrogen compounds in cotton occurs along the positive, dynamic gradient that exist between the high concentration at the point of original formation or digestion of a compound and the low concentration at the point of storage or utilization. The acceleration in observed rate above the calculated rate on the basis of diffusion acting alone is variously assigned to protoplasmic circulation, mass flow of a concentrated solution (1), etc.

Fig. 4. Sugar gradients in silking maize plants at 4 p. m. on July 20, 1934.

The samples for these analyses were taken five days after those for figure 3. The sucrose gradient is changing from positive to negative; reducing sugar gradients continue negative.

Sugar Gradients in Maize

Some 300 samples of leaves, midribs, leaf sheaths, stalks, roots, and ear shanks of the maize plant, selected to show concentration gradients of sugars at various stages in the development of the plant, have been analyzed by the author and his assistants[2]. These analyses have shown that concentration on a diffusion gradient is concerned. Evidence that the

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[2] Especial credit is due Mr. Kenneth Burnett and Mr. C. G. Barr, without whose assistance these analyses could not have been completed.

All analyses were made with samples rapidly killed in boiling alcohol. Sugars were extracted with 80 per cent alcohol and cleared with neutral lead acetate. Sucrose was inverted with invertase and calculated as invert sugar and sucrose. Levulose was determined by Jackson's modification of Osts' copper carbonate method and glucose by difference of levulose and reducing sugars.

sucrose is the principal sugar of the leaves of maize and of the older stem. Sucrose accumulates rapidly in the leaves on a bright day and disappears at night, whereas hexose sugars, glucose and fructose, show a much smaller diurnal variation. The hexoses are relatively high in the young stalk and decrease slightly with age, but the sucrose content of the stalk reaches a maximum at or shortly before maturity, and shows a diurnal fluctuation which lags approximately two hours behind the sucrose curve of the leaves.

Sugar concentration gradients in maize sampled at 4 p. m. in the late silking stage are shown in figure 4. Glucose showed a positive gradient from mid-ribs to leaf sheaths and sucrose was positive from sheaths to stalks. All other gradients are negative and indicate that the total sugar contents of the various tissues through which the sugar was presumably moving were successively higher rather than lower. Our analyses were necessarily made on the entire tissues, of which the conducting cells constitute only a small fraction. If, however, the living cells of the plant are connected by plasmodesms as the work of Martin (13) and others indicates, there should not be a marked lag between the time changes in the sugar content of the phloem and of the adjoining, directly connected parenchyma, unless some factor other than equalization of sugar content of the bulk tissues did vary with translocation is given in the stem sucrose curve of figure 3 and in the stem and ear shank sucrose values in figures 5 and 6. The stem sucrose content shown in figure 3 increased more than 200 per cent between 8 a. m. and 6 p. m., even though the entire stalk, including the pith and rind as well as the vascular bundles, was used for the analyses.

The data from a series of analyses made in the glaze stage when translocation should have been at its maximum, are shown in figure 5. The reducing sugar gradients are the same as those in figure 4, both steeply negative. There is no possible question regarding the sucrose gradient at this stage. It is negative and more strongly so in the early morning when translocation should have struck a balance for the day. The increase of sucrose in the leaf during the day is, of course, due to photosynthesis; the decrease of sucrose in the ear shank at the same time was found only in this experiment and is explained on a basis of temperature. The night of this run was abnormally cool with temperatures below the minimum for growth in maize (7), and apparently the utilization of sucrose within the ear was slowed down at night and speeded up on the bright, warm day. If temperature is taken as a primary factor in the night time accumulation of sucrose in the ear shank, we must assume that translocation is less rapidly affected by low temperatures than utilization and storage. Curtis (2) has shown that translocation is sharply inhibited in Phaseolus at 5° C. The air temperature had dropped to 8.0° C. at 4 a. m. and reached 27.0° C. at 4 p. m. on the day on which these samples were collected. At 10 a. m. the air temperature was 23.5° C. and the sugar content of the ear internode had dropped nearly 20 per cent below its 4 a. m. value, whereas the sugar concentration of the ear shank was maintained. At 4 p. m. the sugar content of both tissues dropped sharply and by 10 p. m. it had risen to the high value of the day, wtih an air temperature of 14.0° C.
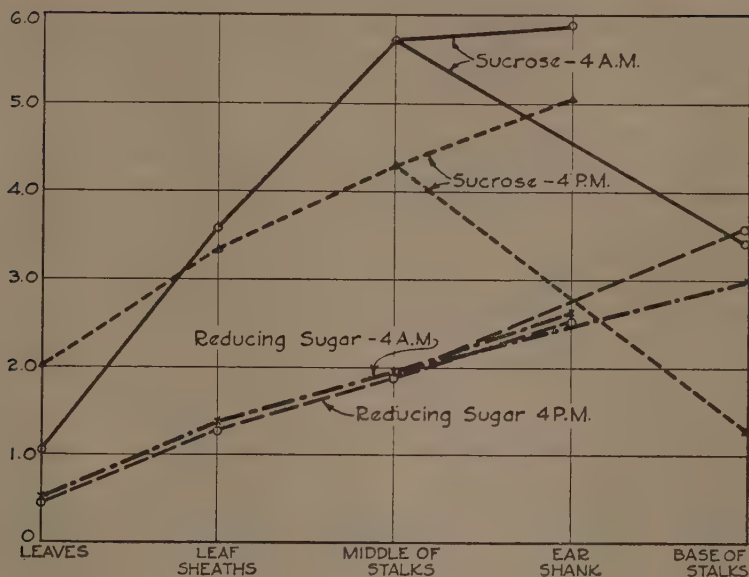
Fig. 5. Sugar gradients in maize plants with well developed ears, sampled August 20, 1934.

All gradients, except those to the base of the stalks, are strongly negative. Sucrose again shows the more pronounced diurnal fluctuations, complicated in this case by the low night temperature of August 19-20.

The Effect of Defruiting

Maskel and Mason (11) found that gross analyses of the bark of cotton gave uniformly negative gradients of organic nitrogenous materials between the upper and lower parts of the plant. By reversing the direction of translocation they showed that the translocation gradient was actually positive but had been obscured by the accumulation of non-moving, nitrogenous compounds in the older bark of the lower stem. Maize does not lend itself to manipulations of the type used with cotton, but it was possible to stop translocation to the ear by removing this organ.

Analyses on defruited stalks are shown in figure 6. These samples were taken from adjoining rows at the same time (August 30, 1933) as the material used for the data of figure 5. Removing the ear stopped the day time drop in the sucrose content of the middle stem, which we take as support of the view that this drop was caused by increased growth and storage in the ear under the influence of higher temperatures. The interesting point in the gradients now becomes the sample from the base of the stalk. Early in the present paper we pointed out that defruited plants showed a superior root growth while the roots of fruiting plants showed evidences of a deficient supply of organic foods. Certainly the removal of the ear should be expected to increase the movement of sugars from

the central to the basal region of the stalk. A glance at figures 5 and 6 will show that if this increased translocation occurred, it resulted in the building up of a negative sucrose gradient within the stalk. There was a strong positive gradient down the normal stalks in which a m i n i m u m of translocation would have been expected, and a negative gradient down the defruited stalk in which translocation should have been proceeding at a maximum rate. The sharp diurnal fluctuation in the sucrose content of the base of the normal stalk (fig. 5) may indicate that sucrose was moved down the stalk with the high positive gradient built up on the cool night, and back to the ear along a polarized gradient, such as apparently was present between the leaves and the ear, when the sucrose content of the ear shank dropped with the higher day time temperature. Complete analyses are not available to show that this sugar did not move into the roots, but their generally poor condition suggested that it did not.
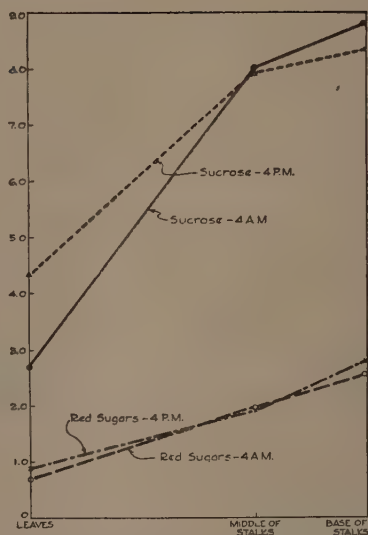
Fig. 6. Sugar gradients in defruited maize plants sampled August 20, 1934.

The negative sucrose gradient extends to the base of the stalk in defruited plants, and the day time drop at the middle of the stalk, shown in figure 5 and assigned to increased growth and storage in the ear with higher temperature, is not present.

DISCUSSION AND SUMMARY

1. When 25 per cent of the leaves of silking maize were left at the base of the plant, the grain yield was less than one-third that of plants with the same leaf area at the top of the stalks. The difference was due to a larger number of developing fruits on the plants with leaves above the ear node. After fertilization and the initiation of rapid translocation toward the ear, the lower leaves were equally as effective as the upper. These results are considered to indicate that the foods elaborated by the lower leaves move downward in the young plant, but that this current is reversed after fertilization when the ear dominates the food supply of the plant; a dominance which leads in extreme cases to premature death of the roots through starvation alone or more commonly with complicating, weakly parasitic diseases. The effect of the developing fruits upon the translocation mechanism could be transmitted mechanically from cell to cell in the phloem, increased activity in one cell with increased and reversed rate of movement affecting directly and successively the metabolism of the adjoining translocating cells; the stimulus could be transmitted

electrically after the scheme of Lund (10), from its origin in the accelerated metabolism of the developing embryos, or it might depend upon the backward movement of a chemical substance of the nature of a hormone. Present evidence does not justify a choice among these hypotheses, although a metabolic stimulus, electrically transmitted, would appear to satisfy the conditions of the reversal of polarity apparently involved.

2.   Translocation rates in maize probably reach a daily peak between 2 p. m. and 6 p. m. and drop to a low rate by 10 p. m. Temperature, through its effect upon growth and possibly through a direct effect upon the activity of the phloem, seems to be an important factor in determining maximum rates. In many of our experiments the sugar content of both the leaves and stem of maize reached a minimum value at about 8 a. m., suggesting that the higher morning temperatures accelerated the translocation and utilization of these substances to a point above the early photosynthesis rates.

3.   The polarity of translocation in the maize plant appears to be a positive force not explainable by any theories of translocation yet advanced. At the time when the ear was gaining most rapidly in weight it showed the strongest negative gradient for both the reducing sugars and sucrose. Starving corn roots showed positive translocation gradients; that is, the sugar content of the roots was lower than that of the central stalk, but the well-nourished roots of defruited plants showed negative gradients. During the period of most rapid translocation, the gradients of total sugar and of all sugar fractions were negative for each tissue between the leaf blade and the cob of the young ear. The positive action of translocation resulted in the piling up of sucrose in the ear shank at night to a value more than five times that of the leaves. Neither mass flow nor any acceleration of movement along a positive translocation gradient can be used to explain such results, and an hypothesis of positive, polarized action within the phloem is suggested.

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LEAF AREA AND GROWTH RATE OF CORN PLANTS[1]

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The effects of different rates of planting upon yield of corn have been investigated by many workers but the effect of such planting upon rate of growth and development of different parts of the individual plants have not been carefully studied. It is the purpose of this paper to present data which show the extent to which rate of planting may modify the development of various vegetative parts of the plant. This work was conducted in 1932 on a private farm near the campus of Iowa State College, by the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.

In these experiments the kernels were planted thickly enough to permit the thinning of each plot to a definite number of plants per hill when the plants were permanently established, or about three weeks old. They were then thinned to one, three, or five plants per hill. For the sake of convenience plots planted at the rate of five plants per hill will be designated as five's, plots with three plants per hill as three's, and those with one plant per hill as one's.

Leaf area, plant height, basal area of the stalks, and dry weight of the plants were determined at weekly intervals from the middle of June to the end of the growing season. Leaf area was determined by multiplying the product of the length and width of the leaf by 0.75. All the leaves of several plants were measured and the average leaf area per plant was determined. Plant height was taken as the distance from the ground to the tip of the highest out-stretched leaf. The basal area of the stalk was determined by measuring the larger and lesser diameters of the first internode above the ground level. These measurements were made with calipers and the product of the two diameters by 3.1416 was considered to be the basal area. In order to obtain dry weight, representative plants, without roots, were brought into the laboratory and dried. The moisture content of the larger stalks was reduced by cutting the weighed stalks into thin strips and placing in the sun under greenhouse glass. After 24 hours the plants were sufficiently dry to permit drying to a constant weight in an electric oven.

EXPERIMENTAL RESULTS

There was no appreciable difference in leaf area of plants in the different rates of planting prior to June 21. On June 21 an average plant in hills planted at the rate of one, three, and five plants per hill had developed respectively 1,061, 1,042, and 1,004 square centimeters of leaf area. By the fifth of July, plants from the same rates of planting showed 4,769, 3,684, and 3,277 square centimeters of leaf area. Or in other words,

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[1] Journal Paper No. J223 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 89.

there was 32 per cent less leaf area per plant in the five's than in the one's, and 24 per cent less in the three's than in the one's.

The maximum leaf area, per plant, in the one's, three's and five's was 8,900, 7,908, and 6,573 square centimeters. In the five's the maximum leaf area was reached on July 25; in the three's on August 8; and in the one's on August 15. After the 25th of July firing of the lower leaves in

TABLE 1. *Leaf area per plant in square centimeters*

Date	Number of plants per hill		
	One	Three	Five
June 21	1061	1042	1004
June 28	4334	2848	2198
July 5	4769	3648	3277
July 12	7101	6102	4572
July 19	7369	5964	5629
July 26	7199	6863	6573
August 1	8639	7152	5010
August 8	8740	7908	5901
August 15	8900	6880	5333
August 22	8240	6689	5026
August 29	6683	5518	4447
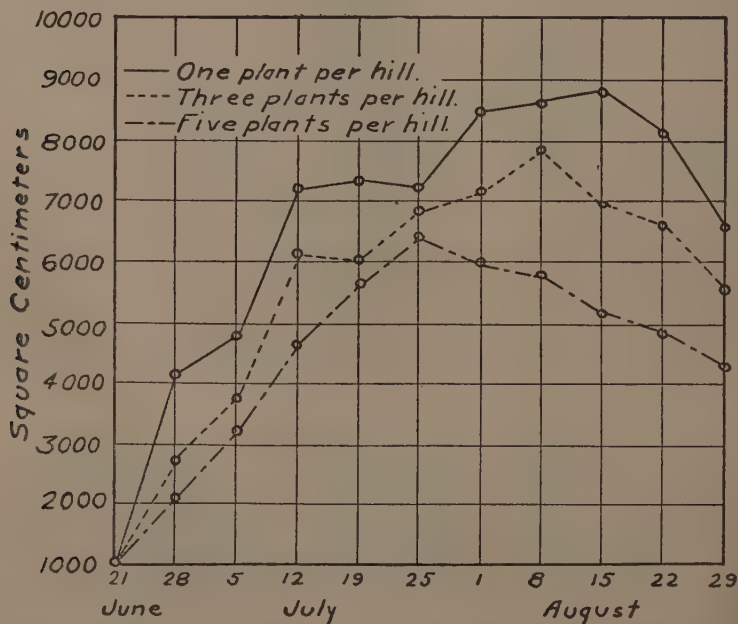
Fig. 1. Comparison of the average leaf area of plants grown in different rates of planting in 1932.

the five's was more rapid than was the formation of new leaf area by elongation. The actual leaf area at weekly intervals is shown in table 1. Figure 1 graphically presents a comparison of the average leaf area of plants grown in different rates of planting.

INCREASE IN HEIGHT

Height of the plants does not seem to be appreciably affected by rate of planting. On June 21 the plants in the one's, three's, and five's averaged 83, 85, and 92 centimeters in height. Since the leaf area per plant at this time is about the same regardless of rate of planting, the slightly greater height in the thicker rates is probably a response to crowding. By the time the plants had reached their maximum height, or about August 10, there was almost no difference in height of the plants in the three rates of planting. This result is shown more clearly in table 2.

TABLE 2. *Height of plants in centimeters*

Date	Number of plants per hill		
	One	Three	Five
June 22	83	85	92
June 29	122	124	130
July 6	158	154	161
July 13	203	189	198
July 20	241	240	249
July 27	269	267	265
August 3	270	268	264
August 10	274	263	272

BASAL AREA OF STALKS

One of the outstanding differences in the development of the plants with varying rates of planting was shown in basal area of the stalk at the ground level. As early as the middle of June when the plants were about one month old, the basal area per plant in the five's was only about 60 per cent as great as that in the one's. At this time there was no appreciable difference in either leaf area or height. By the time the maximum diameter was reached the stalks in the five's were only 45 per cent as large as those in the one's. Data showing the development of the basal area of the stalks are given in table 3.

TABLE 3. *Basal area of average stalks in square centimeters*

Date	Number of plants per hill		
	One	Three	Five
June 22	3.08	2.36	1.95
June 29	7.82	4.49	3.42
July 6	9.49	6.09	4.12
July 13	9.30	5.50	3.77
July 20	8.70	5.50	3.77

An attempt to account for this wide variation in the cross-sectional area of the stalks is beyond the scope of this paper. However, it is interesting to note that since the stalks in the five's were much smaller than in the one's they would be weaker and might break over more easily in a heavy wind.

INCREASE IN DRY WEIGHT

A plant grown at the rate of five plants per hill weighed slightly more on the 22nd of June than a plant grown singly in the hill. By July 5 a plant from the five's weighed less than half as much as a plant from the one's. This reversal occurred three weeks before the maximum leaf area was reached in the five's. By the first of September a plant from the five's weighed only about one-third as much as an average plant from the one's. The total weight of all the plants in the three's was about twice that of a single plant in the one's. An average hill from the five's weighed only a very little more than did an average hill from the three's. There was a close correlation between the dry weight and the basal area of the stalks.

TABLE 4. *Dry weight of single plants, without roots*

| Date | Number of plants per hill | | |
	One	Three	Five
June 22	9.7	10.4	15.1
June 29	37.0	40.2	24.4
July 5	101.7	70.2	47.5
July 12	210.0	162.0	74.0
July 19	270.0	156.0	109.0
July 25	399.0	290.0	179.0
August 1	535.0	303.0	132.0
August 8	563.0	366.0	180.0
August 15	728.0	298.0	222.0
August 22	618.0	350.0	219.0
August 29	762.0	461.0	267.0

Early in the season while the plants were small, it was possible to obtain the dry weight of several plants from each rate of planting. As the plants increased in size, drying facilities became inadequate and fewer plants were collected at each date. Variation in these small samples accounts for the irregularities in dry weight of the larger plants as shown in table 4.

Figure 2 shows, in graphic form, the material presented in table 4. This figure shows that when the dry weight is plotted an autocatalytic reaction curve results. The slope of the curve decreases as the planting rate is increased.

YIELD AND SIZE OF EARS

The length of the ear seems to be appreciably reduced by thicker planting, but the diameter of the ears is not so markedly affected. The dry weight of the ears increased in much the same manner as did the dry weight of the stalks, except that the ears were less mature in the thicker
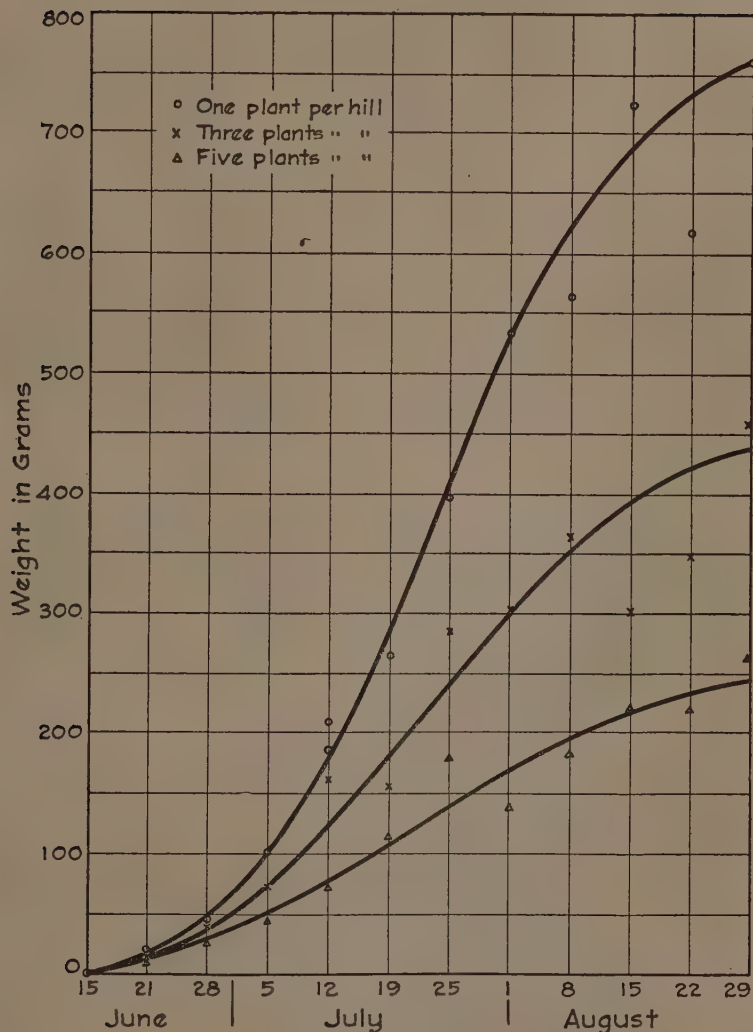
Fig. 2. Increase in dry weight of single plants from three rates of planting during 1932.

planting rates at any one date, because the silks developed more slowly as rate of planting increased. The data in table 5 show yield, number of ears per 100 hills, number of "nubbins" (ears less than 15 centimeters long) per 100 hills, and the average length and diameter of the ears, collected at harvest in October.

TABLE 5. *Comparison of yield, number and size of ears as modified by rate of planting*

Plants per hill	Yield (bu.)	Number of ears per 100 hills		Average length centimeters	Average diameter centimeters
		Total ears	"Nubbins"		
One	45.5	141	17	20.1	4.98
Three	72.5	265	23	18.9	4.85
Five	60.5	359	152	14.9	4.42

The largest yield was obtained from the plots containing three plants per hill, and the smallest where only one plant was left in a hill. These figures agree closely with the figures showing dry weight of the stalks. In the one's every stalk produced one large ear, and half of them produced two ears. In the three's not every plant produced even one ear, and in the five's less than 75 per cent of the plants produced an ear and almost half of these were "nubbins."

SUMMARY

The growth rate of corn plants is modified by rate of planting. An average plant in the five's contained 30 per cent less leaf area by the first of July than one planted singly in the hill. Maximum leaf area was reached at a much later date in thinner planting rates because of the firing of the lower leaves of plants in the thicker rates which began as early as the middle of July. Rate of planting did not modify significantly the height of plants.

The basal area of stalks in the five's on the 22nd of June was only 60 per cent as great as that of plants in the one's. When maximum size was reached the basal area of a stalk from the five's was less than 50 per cent as great as that of the one's.

The dry weight of all plants per hill in the five's was no greater than that of all the plants per hill in the three's and only about twice as much as that of a single plant in the one's.

Every stalk in the one's produced a large ear, and about half of them produced two ears. Not every plant in the three's produced an ear and ears were formed on less than 75 per cent of the plants in the five's. The number of "nubbins" increased with increased rate of planting.

RELATION OF RATE OF PLANTING TO THE EFFECT OF CORN SEED TREATMENT [1]

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Preceding 1923 the major aim of corn seed treatment experiments was to find a fungicide effective in the control of seed-borne diseases, which did not depress the yields of extra good seed. After this was accomplished, attention was directed principally to the testing of numerous satisfactory fungicides in order to single out the superior ones and to determine the proper concentrations of toxic agents. In these earlier tests border effect and rate of planting were not always adequately checked. In 1930 Kiesselbach (3) presented data showing that seed treatment was of little or no value under Nebraska conditions when border effects were eliminated. Clayton (1) and Kiesselbach (3) believe the value of seed treatment is confined to the effects produced by changes in field stands. If the value of seed treatment is merely a matter of increasing the stand then the investigator dealing mainly with stands greater than necessary for maximum production should obtain results showing seed treatment to be depressing to yields, while identical experiments, except that they were conducted under conditions where the same stands would be less than necessary for maximum production, should present results exactly opposite, namely benefits from seed treatments. This led to the initiation of experiments in 1930 designed to throw light on the influence of stand on the effects of seed treatment.

MATERIALS AND METHODS

Commercial dust seed treatments were used at the rate of two ounces per bushel. All experiments were conducted at Ames, Iowa. Harper's strain of Reid's yellow dent corn was used. This strain germinates relatively fast in cold soils and is only moderately susceptible to seedling injury by the dry-rot organisms. Good and poor seed lots were selected. The good seed lots were as nearly disease-free as could be obtained by germinator ear testing and are designated "nearly disease-free" in these experiments. The diseased seed lots were moderately infected (at least 25 per cent of the kernels) and are designated "Diplodia-infected," "Basisporium-infected," and "Gibberella-infected," as the case may be. The spacing was three and one-third feet between rows and between hills in the row, approximately four thousand (3920) hills per acre. The field tests included natural and controlled stands. Natural stands were obtained by planting by hand at the rates decided, namely 2, 3, 4 and 5 kernels per hill. Controlled stands were obtained by planting in excess of the rates named and thinning with putty knives to the desired rates when the plants were about five inches in height.

The plantings were systematic in order, using four-row plots each row 12 hills long and harvesting the two middle rows to eliminate border effects. Each replication consisted of five plots, the first two, paired not treated and treated seed in natural stand, the second two in controlled stand (thinned to stand) and the fifth plot in the replication was a soil check which was always planted at the same rate (three kernels per hill) and with nearly disease-free seed. Therefore, each replication consisted of 20, 12-hill rows and five replications made a range 100 rows long and 12 hills wide. When this was the first range in an experiment it was planted at the rate of two kernels per hill except the soil check plots which were always planted at the rate of three kernels per hill. The next range along-side was planted at the three-kernel rate, the third range at the four-kernel rate, and the fourth range at the five kernel rate. One type of seed was used in this block 100x48 hills, for example "nearly disease-free." Another type was used in a similar block, for example "Diplodia-infected." As many blocks were used as there were types of seed to be tested. The outline of the experiment so far is for five replications of all com-
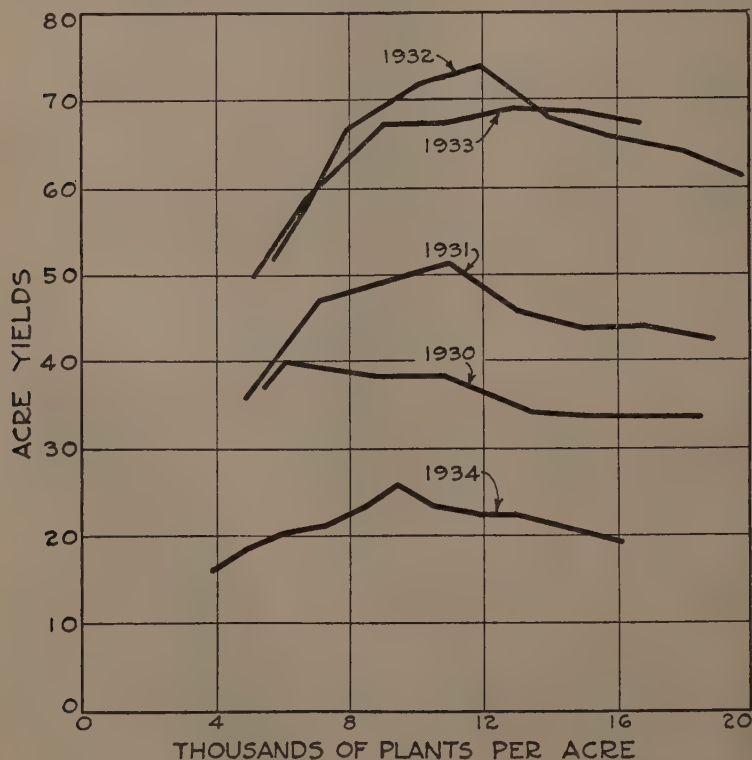
Fig. 1. Relation of field stands (number plants per acre) to acre yields of ear corn, 1930 to 1934.

parisons. Often the five replications were increased to ten by duplicating the outlined plan. The number of replications are shown in the tables. This experiment was conducted for five years, 1930 to 1934, and the results are presented in this paper.

RELATION OF FIELD STANDS TO YIELDS 1930-1934

Field stands were determined by counts when the plants were five to six inches in height and at the same time the plots dealing with controled stands were thinned to the desired stands. This was past the usual seedling blight stage and it was noted that little change in stand occurred after this time.

Stands and yields seemed closely correlated in nearly-disease-free and diseased seed lots so all the comparisons were used in the curve depicting the relation of field stands to yields. The number of comparisons making up the curve for any year varied from 48 to 84 depending on whether each pair was a mean of five or ten replications. These results are shown graphically in figure 1.

Figure 1 shows that the most productive stand varied with the season being as low as 7000 plants per acre (one and three-fourths plants per hill) in 1930 and as high as 13,000 (three and one-fourth plants per hill) in 1933. The plots were not on the same land each year, but they were on the same farm and on land of about the same fertility. In three of the five years the small amount of moisture was a very important factor in yields. For the purposes of this paper it is sufficient to point out that in each year increase in stand was followed by increase in yield up to the most productive stand. Beyond this point an increase in stand was followed by a decrease in yield. If seed treatment affects only the stand then all significant yield increases in this experiment in any year should be in stands less than the most productive one and significant effects, if any, beyond the most productive stand should be decreases.

YIELD DATA

Because the usual size of the experiment was as large as eight acres, it was thought that a soil check in each replication would help in the interpretation of the data. Systematic differences in productiveness of ranges were corrected by this means. The important point to be remembered is that all comparisons of results from not treated and treated seed are from pairs located side by side in the experiment so that, whenever correction was made, both members of the pair were multiplied by the same ratio and, therefore, continued to hold the same relationship to each other.

Table 1 presents the stand and yield data for each of the five years. Table 2 summarizes table 1 by presenting the differences in yields following seed treatment.

In interpreting the data in table 2, those dealing with nearly disease-free seed corn can apparently be eliminated with the statement, similar to those made in the past (2, 4, 5, 6), that this type of seed was neither benefited nor injured by seed treatment. Only under adverse growing conditions (cold, wet weather), shortly following planting, would any benefit be expected. Plantings were made at regular corn planting time

TABLE 1. *Feild stands and yields from not treated and treated Yellow Dent seed corn, ten relications, Ames, Iowa*

Seed corn	No. kernels per hill	Natural stand				Controlled stand			
		Not treated		Treated		Not treated		Treated	
		Stand	Yield	Stand	Yield	Stand	Yield	Stand	Yield
1930									
Nearly disease-free	2	6830	40.0	7010	40.9	7510	42.3	7200	40.4
	3	10350	36.2	10020	37.6	11550	36.3	11480	38.6
	4	13780	32.1	13800	33.3	15320	30.1	15580	31.7
	5	17460	32.1	17500	32.3	18580	28.6	18980	32.3
Diplodia-infected	2	4935	33.8	5780	40.4	6870	40.5	7340	43.2
	3	6555	34.3	7820	37.3	10610	40.2	10920	39.2
	4	9640	36.6	11630	40.0	14050	34.8	15100	32.8
	5	11620	34.7	14380	38.9	17140	36.9	18380	33.5
Basisporium infected	2	5630	37.4	6380	42.6	6970	42.4	7170	41.2
	3	8150	42.6	8640	40.2	10440	37.4	11000	39.8
	4	11500	39.1	12380	34.3	14220	32.8	14930	32.9
	5	13980	38.5	15850	33.9	18400	34.2	18600	34.2
Gibberella infected	2	6300	35.0	6910	39.7	7300	40.1	7650	41.2
	3	8280	34.4	9920	36.7	10450	38.9	11180	37.6
	4	12720	32.9	13760	34.3	14770	31.7	15380	31.4
	5	15740	31.2	17050	32.1	18510	39.0	18840	38.6
1931									
Diplodia-infected	2	4375	32.2	5310	38.8	7595	46.4	7690	49.0
	3	6940	41.8	8780	45.8	11070	47.8	11600	52.0
	4	8920	45.0	11330	47.9	15070	44.8	15560	40.9
	5	10090	47.2	13930	51.0	18250	41.9	19200	40.6
Gibberella infected	2	6010	41.1	6120	43.3	7400	48.1	7350	46.7
	3	8960	52.1	9500	53.3	11500	53.8	11690	48.8
	4	12900	45.6	12600	46.1	15440	41.5	15280	45.5
	5	15590	41.5	15780	45.7	19400	42.8	19270	42.7
Nearly disease-free	2	6875	48.1	6695	49.0	7760	53.5	7560	51.7
	3	10280	52.8	10075	54.8	11430	52.2	11300	53.0
	4	13880	43.3	13500	44.2	15330	43.9	15090	43.2
	5	16890	42.1	17080	45.5	18850	43.2	18850	42.8
1932									
Diplodia-infected*	2	6277	55.5	6800	57.8	7966	67.6	8000	66.6
	3	9500	66.9	10400	75.3	11933	79.4	11900	77.5
	4	12267	65.4	13783	68.4	16000	70.4	16000	68.7
	5	15267	60.1	17867	59.4	19933	65.7	20000	59.0
Basisporium infected	2	6883	63.3	7016	65.3	7883	68.5	7983	71.2
	3	10167	73.2	10833	75.2	11900	76.2	11967	77.9
	4	13983	73.1	14616	72.9	15950	74.9	15983	73.7
	5	17217	68.1	17583	62.4	19817	66.1	19933	62.3
Nearly disease-free	2	6767	57.2	6866	58.6	7900	64.6	7950	64.2
	3	10383	71.1	10517	74.3	11967	79.8	11867	77.4
	4	14016	68.0	14433	65.9	15483	68.9	15850	66.5
	5	17783	67.9	18350	62.9	19600	65.5	20000	62.2

TABLE 1. *Continued*

	No. kernels per hill	Natural stand				Controlled stand			
Seed corn		Not treated		Treated		Not treated		Treated	
		Stand	Yield	Stand	Yield	Stand	Yield	Stand	Yield
1933									
Basisporium-infected	2	5827	43.3	6640	47.9	6640	49.4	6910	52.7
	3	9205	60.6	9777	65.3	9550	62.4	9950	64.5
	4	11640	63.8	13100	74.2	12700	72.2	12240	69.2
	5	14470	63.8	15430	68.7	16210	69.5	16900	72.4
Diplodia-infected	2	5010	43.1	5655	48.2	6990	59.8	6940	60.2
	3	8400	62.6	8910	66.0	10220	70.4	10000	69.7
	4	11040	63.0	11500	60.6	13580	64.5	13580	65.1
	5	13520	63.6	14810	64.5	16880	64.7	17100	63.8
Nearly disease-free	2	4520	52.7	4350	49.2	6025	63.8	5630	62.8
	3	6800	70.2	7140	69.0	8820	72.6	8890	74.7
	4	8890	75.9	8870	72.0	12640	72.9	12480	71.6
	5	10880	74.8	11230	71.8	15500	74.4	15650	71.6
1934**									
Diplodia-infected	2	3300	13.3	4716	17.4	5083	17.4	5083	20.6
	3	5200	16.5	7117	20.4	7633	18.5	7633	21.2
	4	7367	15.9*	11267	24.2*	10400	21.4*	10400	20.8*
	5	8533	17.4*	13430	24.4*	11917	22.5*	11917	19.7*
Basisporium-infected	2	5467	17.6	4433	18.2	4933	17.5	4933	20.3
	3	7683	21.1	7883	23.8	8616	22.6	8616	25.2
	4	10900	27.4*	10467	25.9*	11167	22.0	11167	21.1
	5	13067	25.9*	16333	29.5*	13150	21.1	13150	19.5
Nearly disease-free	2	5433	20.2	5067	20.2	5817	20.9	5817	23.0
	3	7500	26.3	9783	31.0	9350	26.1	9350	27.9
	4	9100	22.2	11800	25.9	10645	22.7	10645	24.5
	5	11283	16.9	15283	17.7	13621	16.3	13621	18.0

* Only five replications.
** Due to drought at planting time such poor stands were obtained that stands could not be controlled to the desired rates and, therefore, were thinned to equal that of the poorer plot in each pair.

and, in these five years were not followed by adverse conditions unless the extreme drought of 1934 be considered as such. Germination of seed corn was delayed in 1934 by lack of moisture and under these conditions the eight comparisons each have an average of ten replications, show seven increases following seed treatment of nearly disease-free seed and no decreases (fig. 2). These results indicate that any condition militating against rapid germination gives greater opportunity for benefit from seed treatment.

The most striking results shown in table 2 are those with Diplodia-infected seed. The experiments dealing with natural stands resulted in 18 yield increases in 20 trials, while those dealing with controlled stands resulted in only seven yield increases in 20 trials. The contrast
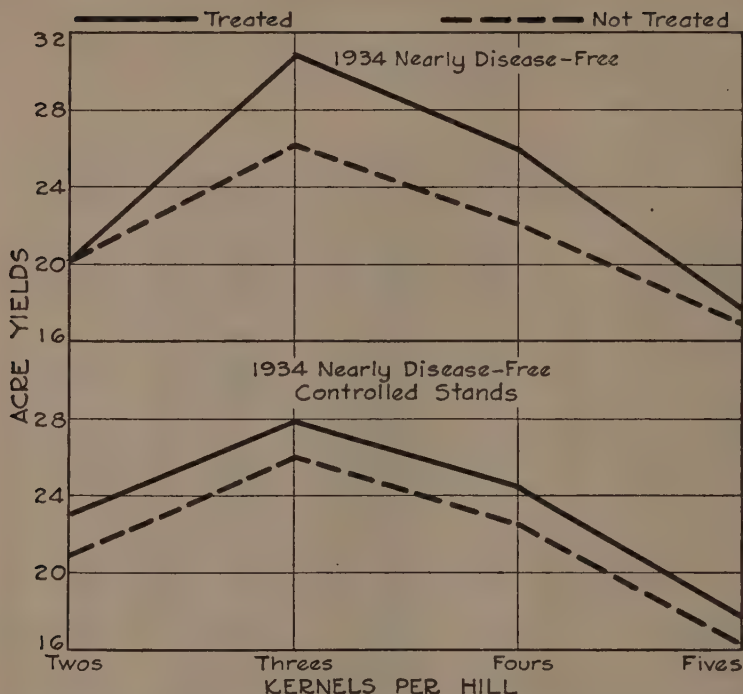
Fig. 2. Effect of seed treatment on acre yields from nearly disease-free seed corn planted at rates 2 to 5 kernels per hill or thinned to 2 to 5 plants per hill (controlled stands), 1934.

is even greater when only the thicker rates of planting (fours and fives) are considered. Two out of ten trials show yield decreases in the natural stands, but nine out of the ten show decreases in the controlled stand.

The natural interpretation, if only the data dealing with controlled stands were at hand, would be injury by the treatment. In this case the decreases in yields could not be caused by excessively thick field stands because the stands, from treated and not treated seed were made equal by hand thinning.

If the seed treatment was injurious to Diplodia-infected seed, it should have the same effect under conditions where increases in stand would not cause increases in yield. But, under natural stand conditions, there were average increases of three bushels per acre in the high rates of planting (fours and fives) table 2 and fig. 3.

If other reasons, besides injury by seed treatment, are to be sought as an explanation of the results of experiments with controlled stands of Diplodia-infected seed, they must be connected with methods of controlling the stands. An excess of kernels was planted and the excess was larger in the case of Diplodia-infected seed than other types because even viable Diplodia-infected seed often does not emerge unless treated. As was previously stated, about 25 per cent of the kernels in this type

TABLE 2. *Summary of the data in table 1*

Stand	Seed corn	Year	No. rep.	Increases in bushels per acre				Mean increase	
				Twos	Threes	Fours	Fives	bu.	percentage
Natural	Diplodia-infected	1930	10	6.6	3.0	3.4	4.2	4.3	12.3
"		1931	10	6.6	4.0	2.9	3.8	4.3	10.4
"		1932	10[1]	2.3	8.4	3.0	—0.7	3.3	5.2
"		1933	6	5.1	3.4	—2.4	0.9	1.8	3.0
"		1934	10	4.1	3.9	8.3	7.0	5.8	36.9
		Ave.		4.9**	4.5**	3.0	3.0		
"	Basisporium infected	1930	10	5.2	—2.4	—4.8	—4.6	—1.7	—4.2
"		1932	10	2.0	2.0	—0.2	—5.7	—0.5	—0.7
"		1933	6	4.6	4.7	10.4	4.9	6.2	10.6
"		1934	10[2]	0.6	2.7	—1.5	3.6	1.4	5.9
		Ave.		2.5	1.4	0.8	—0.4		
"	Nearly disease-free	1930	10	0.9	1.4	1.2	0.2	0.9	2.6
"		1931	10	0.8	2.0	0.9	3.4	1.8	3.8
"		1932	10	1.4	3.2	—2.1	—5.0	—0.6	—0.9
"		1933	6	—3.5	—1.2	—3.9	—3.0	—2.9	—4.2
"		1934	10	0.0	4.7	3.7	0.8	2.3	10.7
		Ave.		—0.1	2.1	0.0	—0.5		
Controlled	Diplodia-infected	1930	10	2.7	—1.0	—2.0	—3.4	—0.9	—2.4
"		1931	10	2.6	4.2	—3.9	—1.3	0.4	0.9
"		1932	10[1]	—1.0	—1.9	—1.7	—6.7	—2.8	—4.0
"		1933	6	0.4	—0.7	0.6	—0.9	—0.2	0.2
"		1934	10	3.2	2.7	—0.6	—2.8	0.6	3.1
		Ave.		1.4	0.7	—1.5	—3.0*		
"	Basisporium-infected	1930	10	—1.2	2.4	0.1	0.0	0.3	0.9
"		1932	10	2.7	1.7	—1.2	—3.8	—0.2	—0.2
"		1933	6	3.3	2.1	—3.0	2.9	1.3	2.1
"		1934	10	2.8	2.6	—0.9	—1.6	0.7	3.5
		Ave.		1.5	1.8	—1.0	—0.5		
"	Nearly disease-free	1930	10	—1.9	2.3	1.6	3.7	1.4	4.2
"		1931	10	—1.8	0.8	—0.7	—0.4	—0.5	—1.1
"		1932	10	—0.4	—2.4	—2.4	—3.3	—2.1	—3.0
"		1933	6	—1.0	2.1	—1.3	—2.8	—0.8	—1.1
"		1934	10	2.1	1.8	1.8	1.7	1.9	8.6
		Ave.		—0.6	0.9	—0.2	—0.2		

[1] 10 replications of rates two and four and five replications of rates three and five.
[2] Five replications of rates four and five.

* = 2.776—significant—computations on data from Diplodia-infected seed only.
** = 4.604—very significant.

of seed was infected and viable. Therefore, a higher percentage of the plants at thinning time would be from disease-free seed in the plots with untreated seed than in the plots with treated seed and after thinning the same thing would be true. In these equal stands, we would be testing plants from not treated almost disease-free seed against plants from treated seed, one-fourth of which was infected with *Diplodia zeae*. Un-
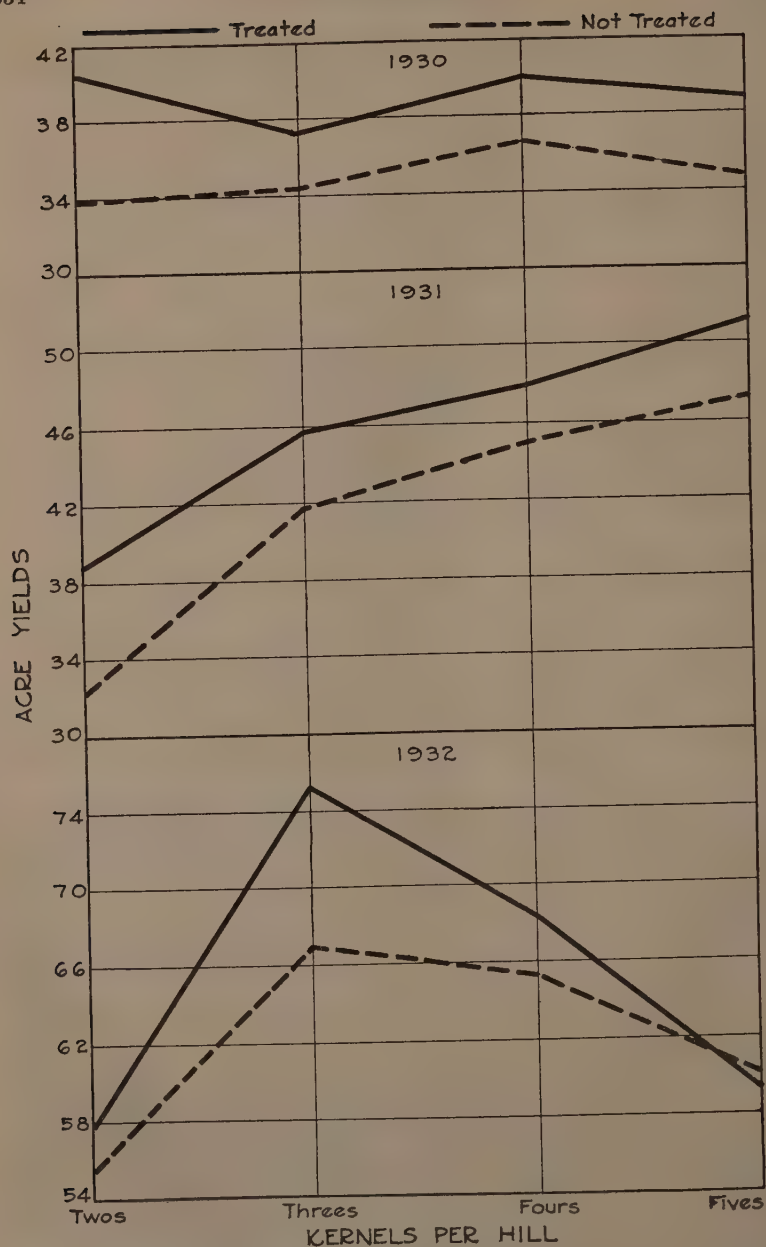
Fig. 3. Acre yields from treated and not treated Diplodia-infected seed corn planted at four rates, 1930-1934.
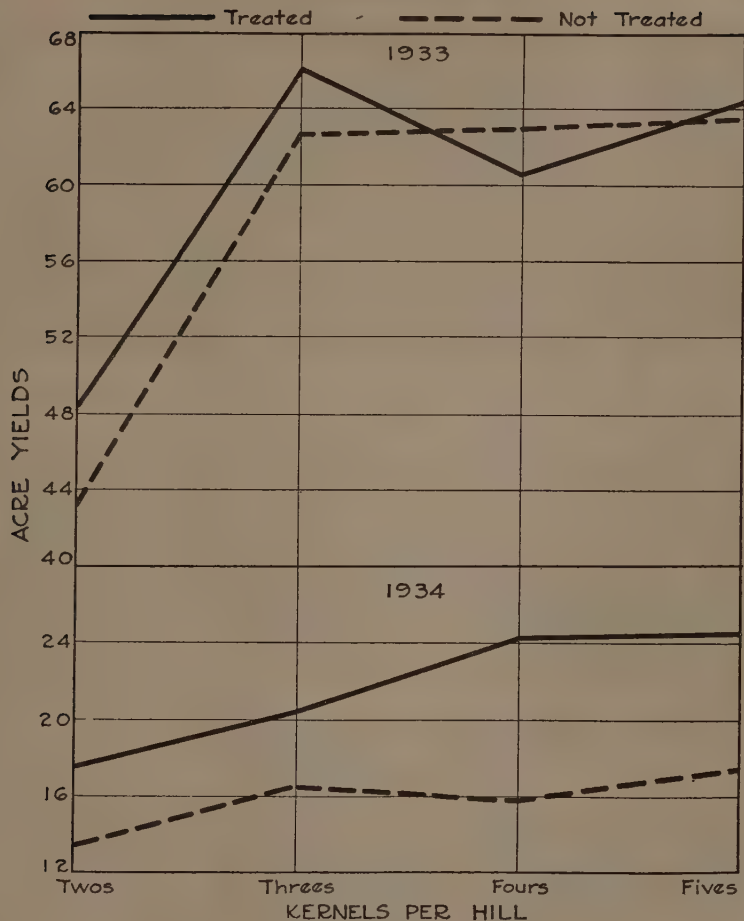
Fig. 3. (Continued)

published data [2] indicate more Diplodia crown infection in plots from treated Diplodia-infected seed than from not treated Diplodia-infected seed when the readings were made after maturity of the corn.

A more apparent criticism of the technic is the fact that there were more plants to be thinned out of the treated hills than out of the hills from not treated seed, and, therefore, more chance for injuring the

[2] Iowa Purnell project 93. Study of Diplodia dry rot of corn. I. E. Melhus and George L. McNew.

remaining plants in the hill. As a matter of fact the hills from not treated seed were often not disturbed at all because they contained the correct number or less of the plants desired.

In seed treatment experiments, cases of injury are usually interpreted from the performance of nearly disease-free seed. In this case, however, less thinning was required in the nearly disease-free plots because a smaller excess of seed was planted and, therefore, less injury was caused. Also favorable conditions followed planting four years out of the five so that nearly an equal number of plants emerged from the not treated and the treated seed.

Therefore, whatever injury resulted from thinning the plots planted with nearly disease-free seed, the injury was equal in the plots that were to be compared with each other. Since the question involved, as will be shown later, can be answered with the data on natural stands, the data dealing with controlled stand will not receive further consideration.

EFFECTS OF SEED TREATMENT BELOW AND ABOVE THE MOST PRODUCTIVE STAND

The relation of field stands to yields of corn have been shown in figure 1. The relationship appears simple in that, starting with low stands, the stands and yields are positively correlated up to a certain point but beyond this point of most productive stand, the stands and yields are negatively correlated, the higher the stands, the lower the yields. The most outstanding effect of a good seed treatment is to increase the field stands from diseased seed. But an increase in stand, in itself, we have just shown, may increase or decrease yields depending on whether or not the increase is in a field stand (1) less than or (2) more than the most productive one. Therefore, to find out, for Iowa conditions,

TABLE 3. *Acre yields of treated and not treated plots with natural stands*

Stand	Seed corn	Paired exp. no.	Not treated bu.	Treated bu.	Increase	
					bu.	percentage
Lower than the most productive ones	Diplodia	17	49.9	53.8	4.4	8.8**
	Basisporium	12	46.3	49.6	3.3	7.1**
	Gibberella	3	42.7	45.4	2.7	6.3
	Nearly disease-free	17	47.0	48.1	1.1	2.3
Higher than the most productive ones	Diplodia	7	53.3	55.8	2.5	4.7*
	Basisporium	8	45.5	44.2	—1.3	—2.9
	Gibberella	5	37.1	39.0	1.9	5.1*
	Nearly disease-free	11	45.6	44.9	—0.7	—1.5

* Significant.
** Very significant.

whether or not the effect of seed treatment is confined to its effect on stand, it is only necessary to assemble the data for these five years with reference to the most productive stand in each year. In general the data should show yield *increases* from diseased seed where stands were lower than the most productive and yield *decreases* where stands were higher than the most productive. These data are presented in table 3.

The data in table 3 do show yield increases where stands were lower than the most productive but the data do not show significant decreases where stands were higher than the most productive. On the contrary, significant increases are shown for two of the three types of diseased seed used which strongly indicate that the effect of corn seed treatment was not confined to its effect on field stands. It would seem, therefore, that seed treatment inhibited or delayed the action of the seed-borne parasite so that more corn was produced on a plant from treated diseased seed than on one from diseased seed not treated.

SUMMARY

Seed treatment experiments, including nearly disease-free, Diplodia-infected and at times Basisporium-infected, and Gibberella-infected seed corn, were conducted during the period 1930 to 1934 at Ames, Iowa.

Not treated and treated seed planted at four rates were compared in natural stands and controlled stands (thinned). In all there were 522 paired four-row plots 12 hills long, of which only the two middle rows (24 hills) were used, thereby eliminating border effects.

These comparisons showed that artificial thinning may injure yields and, therefore, introduce an uncontrolled factor into the experimentation. In the case of plots from Diplodia-infected seed corn, the treated plots were handicapped in performance because they suffered the major portion of the thinning operations.

The seasons, during which these experiments were conducted, were mainly favorable for corn both at planting time and during maturity. These factors are conducive to the smallest possible benefits from seed treatment.

Benefits from seed treatment were greater in comparisons with field stands less than the most productive ones being 4.4, 3.3, 2.7, and 1.1 bushels per acre, than with stands greater than the most productive ones, 2.5, —1.3, 1.9, and —0.7 bushels per acre.

These latter differences show two mathematically significant increases and no significant decreases which indicates that the plants from treated dieased seed were more productive than those from not treated diseased seed. The increases could not have come from increasing the stands because the comparisons are within stands where an increase should produce a decreased yield (see figs. 1 and 2).

Seed treatment killed, inhibited, or delayed the action of the seed-borne parasite so that plants from treated Diplodia- or Gibberella-infected seed outyielded plants from similarly diseased seed not treated and these data strongly indicate that corn seed treatment was not limited to its effects on field stands.

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GENETIC INVESTIGATIONS OF BACTERIAL WILT RESISTANCE IN CORN AS CAUSED BY BACTERIUM STEWARTII (SMITH) MIGULA[1]

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This paper is a preliminary report on the method of inheritance of resistance to bacterial wilt in maize. Twenty-three inbreds of dent corn, one of white flint corn, eighteen of white sweet corn (Evergreen), and fifteen of very early sweet corn, mostly yellow, have been subjected to severe tests for resistance. Crosses between very resistant dent inbreds and very susceptible early yellow sweet and white flint inbreds are being used for inheritance studies. All the inbreds tested were inbred eight or more generations and gave consistent and specific types of reactions. Distinct differences in external symptoms, and morphological disturbances in the normal development of the vascular bundle in certain lines were noted.

METHODS

All plantings of inbreds and F_1 crosses were made in paired rows of which one was inoculated and the other used as a check. Tests made in the greenhouse were repeated in the field. The method of inoculation was essentially as follows: Each individual plant was inoculated with a heavy suspension of bacteria (2,000,000 per cc. or more) by means of a hypodermic needle, through a single puncture in the vicinity of the first node with permanent root system. Greenhouse inoculations were made somewhat earlier than field inoculations, usually about six or eight days after emergence.

The original culture of *Bacterium stewartii* used traces back to a culture (S15) isolated from sweet corn in New York by W. H. Burkholder in 1932. High virulence was maintained by passing the organism repeatedly through a susceptible host. The actions of the reisolations each time were tested on four standard lines of known reaction (two resistant and two susceptible, inbred for 10 generations). By such methods a more or less constant action of the organism was maintained.

RESULTS OF INBRED TESTS

SYMPTOMS

Symptoms of this disease have been reported in detail by Smith (7), Stewart (8), Rand and Cash (4) and Ivanoff (3). These reports for the most part were made from observations on open pollinated varieties and present considerable variation. Field observations made on the various

inbred lines tested in this investigation showed no symptoms not heretofore described but demonstrated that certain symptoms are characteristic of certain lines. All the inbred lines were sufficiently stable to give consistent and specific types of reaction in either field or greenhouse test.

The first characteristic symptom of the disease found on all the inbred lines tested, dent or sweet, is the appearance of water soaked and discolored stripes along the veins of the leaf. These first show up, often within 36 hours after inoculation, as a yellowing of the vein and a slight discoloration in the parenchymatous tissue on both sides. Soon, however, water soaked or irregular shaped semicircular areas begin to appear on either side, which subsequently coalesce into an irregular water soaked stripe extending the full length of the vein. The degree of this leaf striping shortly after inoculation varies with the host line but is in no way an indication of the degree of resistance or susceptibility. For example, Pr a dark green, very resistant field corn inbred consistently shows wider stripes in its leaves than a very susceptible inbred of Golden Bantam or an equally resistant inbred of field corn. On leaf symptoms alone resistant and susceptible inbred lines cannot be differentiated. Usually about two weeks after the first symptoms appear distinct differences in reaction can be noticed. By this time the very resistant lines begin to show signs of recovery whereas the very susceptible ones are either dead or showing a diffuse type of wilt.

On very resistant inbred lines the water soaked streaks begin to dry up within five or six days and remain as scars on the respective leaves throughout the life of the plant. Subsequent leaves are free from any signs of infection and the plant, with the exception of a slight set back in growth, appears to be growing normally. At maturity, stunting can seldom be detected between inoculated and uninoculated paired rows of these very resistant lines. Repeated inoculations of the same plant indicate that no immunity is built up. Other inbred lines less resistant react similarly but show consistent degrees of stunting under similar conditions. Apparently in these stunted lines the organism is not checked as readily in the early stages of growth. This is indicated to a certain extent by histological investigations which have shown a few of the vascular bundles of the more severely stunted lines to remain infected throughout their growth period.

Another reaction characteristic of several of the more susceptible or intermediate inbred lines of sweet corn is expressed as a delayed action of the organism. The plants of these lines in all outward appearance seem to have temporarily recovered without dwarfing, but later at about the silking stage all leaves simultaneously wilt and dry up. Examinations of cut stalks readily showed infection in all the vascular bundles. It would seem that in this case the conditions for active growth of the bacteria were not favorable until the earing stage. This is in direct contrast to those severely stunted lines in which conditions seem to have become more unfavorable as the plant grew older.

On the leaves of the susceptible Golden Bantam inbreds, 797 and 799, the numerous, parallel, narrow streaks appearing shortly after inoculation, gradually unite, two or more at a time, into a wide, brown, irregular stripe of dead tissue extending the full length of the leaf. Such a leaf gives a variegated appearance of brown, pale green, and normal green parallel

stripes. In time more and more of the stripes of dead tissue coalesce usually from the margins inward until the whole leaf blade is involved. The leaf blades usually die off one after another until all are dry while the stem is still green. This type of reaction is distinctly different from the diffuse type found on the very susceptible inbreds W-134 and 887. Very little of the characteristic streaking was evident on the leaves of these lines which turn a pale green, wilt and dry up shortly after inoculation.

HISTOLOGICAL INVESTIGATIONS

Histological investigations have shown the organism to be present chiefly in the protoxylem, trachea and tracheids of infected vascular bundles. Never were traces of the organism found in the phloem. Cross-sections made from seedlings of very resistant lines shortly after inoculation show approximately 10 per cent of the bundles infected, whereas these same lines when sectioned two months after inoculation very seldom show infection in any of the bundles. Sections from inbred lines having a tendency to be stunted indicate that a number of the bundles remain infected for a considerably longer period; often as many as 10 per cent are still infected at maturity. In very susceptible plants from 90 to 100 per cent of the bundles show various degrees of infection. In the more advanced stages the majority of them with the exception of the phloem are completely plugged.

Differences in reaction of the bundles to bacterial invasion under field conditions have been found in the different susceptible lines. Certain of the Golden Bantam and White Flint inbreds have shown a modification in development of the bundle after the protoxylem vessels become infected (Plate I). Instead of the thin-walled parenchyma cells which normally fill in the area between the first annular and spiral vessels and the bundle sheath, there are heavily lignified cells radiating out in all directions from the point of infection. These show a definite radial arrangement and appear very similar to the lignified cells in the bundle sheath. Apparently the invasion of the protoxylem has stimulated the surrounding cells. Some of them appear to be transformed into tracheids, new conducting elements, others merely as lignified cells walling off this infected area from the rest of the plant. No bacteria have ever been found in these cells.

Such a condition has never been found at any stage in W-134, a very susceptible line showing a diffuse type of wilting shortly after inoculation. The Golden Bantam inbreds, 797 and 799, which show this modification have different symptoms and die off considerably slower, leaf by leaf.

CLASSIFICATION ON THE BASIS OF RESISTANCE OR SUSCEPTIBILITY

The 56 inbred lines tested can readily be classified into 5 classes on the basis of resistance or susceptibility as follows:

1. *Very resistant.* Lines that show characteristic streaking in the leaves shortly after inoculation, but readily recover without any visible stunting at maturity.

2. *Resistant.* Those lines which are slightly stunted at maturity otherwise reacting similarly to the above.

3. *Medium.* Lines which are readily stunted from 25 to 50 per cent in their growth, or in the case of early yellow sweet corn, those lines which apparently grow to normal height but dry up shortly after silking.

4. *Susceptible.* All lines in which the plants gradually die, one leaf at a time. All leaves are dead usually within four or five weeks after inoculation.

5. *Very susceptible.* Those lines which have a diffuse manner of wilting, usually dying within 10 to 14 days after inoculation.

Certain of the inbred lines in such a classification are on the border line but the majority of them can readily be placed in the respective classes. A classification of the various lines tested is given in figure 1.

It is evident (fig. 1) that by far the majority of the field corn lines are resistant, whereas the majority of the early sweet corn lines are susceptible. The lines of the Evergreen group are mostly intermediate. This is exactly what one would expect from tests made with open pollinated varieties (Rand and Cash (4)) which have shown field corn varieties, with the exception of the early flints, to be fairly resistant, the Evergreen varieties of sweet corn next in line, and the early varieties of yellow sweet and flint corn as a rule to be very susceptible. Practically all of the inbreds obtained from varieties of Golden Bantam or closely related types have been found to be susceptible. Two of the four lines classed as intermediate are known to be segregates from field-sweet corn hybrids. The other two resemble these very closely and are possibly of the same origin. All four, therefore, may have received their resistance from field corn. The only resistant line within the early group is an inbred of the Black Mexican variety which itself is fairly resistant.

RESULTS OF F_1 TESTS

Reciprocal crosses were made between the two extremes, the most resistant dent corn inbreds and the most susceptible early yellow sweet corn or white flint inbreds. No differences in reciprocal crosses were apparent in the F_1 (table 1). In every case the behavior of the hybrid consistently compared very favorably with the behavior of the resistant inbred line. In certain instances there appeared to be a slight amount of stunting in mature plants in the field, but for the most part this was negligible when compared to the reaction between inoculated and non-inoculated rows of the resistant inbred lines.

In the greenhouse where inoculations were made shortly after emergence the F_1's were usually less stunted than the respective resistant lines involved. The F_1 of OSF X White Flint showed a reduction in dry weight due to the organism of 16.5 per cent, 28 days after inoculation, based on differences between paired rows of 84 plants each. The resistant parent OSF, in the same environment showed a reduction of 24.8 per cent, while the very susceptible parent, White Flint, showed a reduction of 90 per cent. Another test of OSF in which dry weight was taken 20 days after inoculation showed a reduction of 27.3 per cent. This last figure is based on the differences between inoculated and uninoculated paired rows involving 396 plants each. From all indications as the plants reach maturity

Field Corn Inbreds

1	2	3	4	5
	B$_2$			
	KR			
	Lat			
	Cl	Las		
	Ids	Idt		
	Ose	Kb		
MCC	Baw	Ldg	WAL	
Pr	Bls	Se	Mc	
Osf	Tr	La	Ide	STO

White Sweet Corn Inbreds

1	2	3	4	5
		812		
		810	879	
		804	Y-30	
	835	837	883	
	858	826	877	
	855	821	832	
	848	819	818	887

Early Sweet Corn Inbreds

1	2	3	4	5
			910	
			799	
			797	
		899	912	
		898	894	
		1351	G540	GB-16
	BM	1339	906	W-134
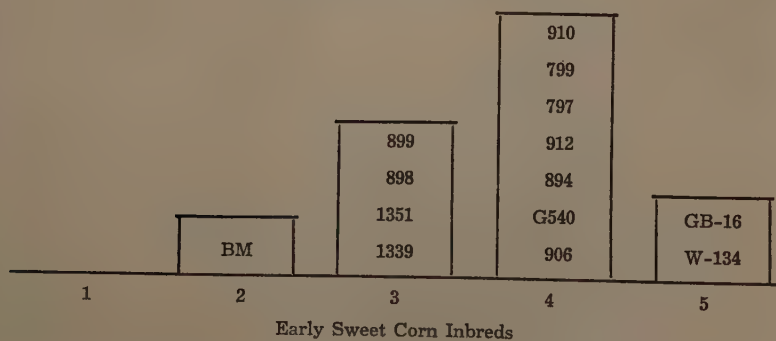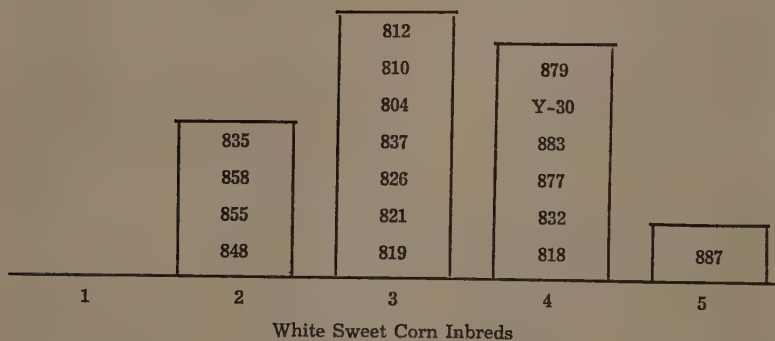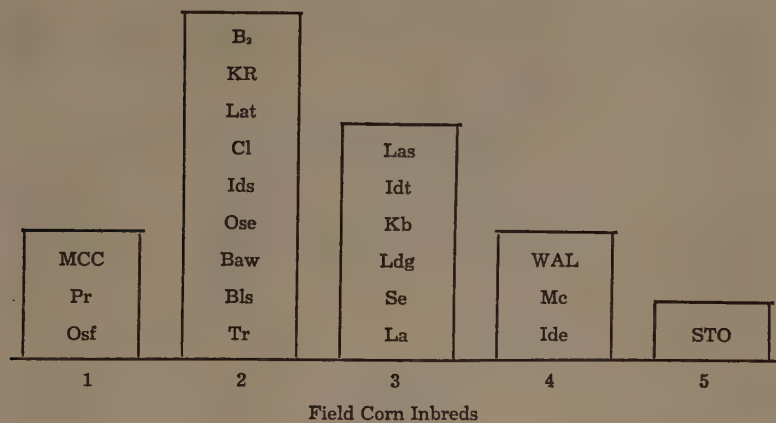
Fig. 1. A classification of inbreds on the basis of resistance and susceptibility.

these differences would gradually disappear. As yet no inbred line or hybrid has been found to be immune. The nearest thing to immunity is a cross of two dent corn inbreds OSF X MCC which shows the highest resistance of all the material tested.

In all, 22 different crosses between inbreds showing various degrees of resistance and very susceptible inbreds showing no resistance have been tested. Resistance proved to be dominant in every case. A few crosses of inbreds where both parents showed a slight degree of resistance have given indications of complementary action of resistance factors. These results will be verified in the near future with tests of a larger number of such crosses. The F_1's of very susceptible inbreds, regardless of any increase in vigor, were very susceptible.

TABLE 1. *The reaction of inbreds and F_1's of resistant X susceptible lines and reciprocals*

Index No.	Inbreds or crosses		Resistance class	Remarks
W-276	Osf (Dent)		1	Very resistant
W-317	MCC "		1	
W-269	Tr "		1-2	
W-515	Pr "		1	
W-473	W.F. (White Flint)		5	Highly susceptible in seedling stage —less as plant grows older
W-544	GB (Golden Bantam)		4-5	Suspectible at all stages of growth
W-1069	W134 " "		5	Most susceptible line
W-866	Osf (1)	x W134 (5)	1	
W-1026	W134 (5)	x MCC (1)	1	
W-874	Osf (1)	x GB (4-5)	1-2	
W-772	GB (4-5)	x Osf (1)	1-2	
W-812	W.F. (5)	x Osf (1)	1	
W-1004	Osf (1)	x W.F. (5)	1	
W-594	Pr (1)	x GB (4-5)	2	
W-775	GB (4-5)	x Pr (1)	2	
W-577	Tr (1-2)	x GB (4-5)	2	
W-889	MCC (1)	x GB (4-5)	2	
W-761	GB (4-5)	x MCC (1)	2	
W-692	MCC (1)	x Osf (1)	1+	
W-985	W.F. (5)	x GB (4-5)	4	
W-475	GB (4-5)	x W.F. (5)	4	

RESULTS OF F_2 AND BACKCROSS TESTS

Inheritance studies have been chiefly confined to backcrosses and later-generation progenies of the crosses OSF X WF and OSF X W-134 (table 1). The backcrosses and F_2 progenies of OSF X WF have been tested in the greenhouse with both highly virulent and attenuated cultures. Plants were classified as apparently healthy or dead. Inoculations with the attenuated cultures resulted in a 3:1 ratio (3 App. H. : 1 dead) in the F_2 and a 1:1 ratio in the backcross, whereas inoculations on the same cross with the highly virulent culture resulted in a 9:7 ratio in the F_2 and a 1:3 in the backcross. With either culture approximately one-

fourth of the backcross progenies died within the time required to kill all the plants of the susceptible parent included as a check. The backcross progenies of (OSF X W-134) X W-134 tested under field conditions with the highly virulent culture likewise resulted in a 1:3 ratio with one-fourth of the progenies dead at a time when all the plants of W-134 (check) were dead. No linkage to the sugary gene was evident.

The above results strongly indicate the existence of four equal classes in the backcross which might be designated as follows: non-resistant, partially resistant, resistant and very resistant. This suggests that at least two major dominant complementary genes are involved, one of which reacts to an attenuated culture. A third gene of less importance may exist. The progenies of 48 selfed plants of the backcross (OSF X W-134) X W-134 recently tested in the greenhouse on two different benches (Nos. 1 and 2), in general, bear out such a hypothesis.

On bench No. 1, which had previously been used for inoculation studies, the disease was much more severe. The progenies of 34 backcrossed plants (13 died early) were from 91 to 100 per cent dead when final classifications were made, leaving 14 progenies segregating for resistance and susceptibility. This is exactly what would be expected on the bases of the above results. However, the remaining 14 (approximately one-fourth) instead of segregating on the basis of a 9:7 as might be expected, could be divided into two classes, one with a close range around 56 per cent dead, the other around 81 per cent dead. This would indicate that there may be a third gene involved which was not differentiated under conditions of previous F_2 and backcross tests.

The reactions on bench No. 2 were what one might expect with an attenuated culture. Approximately one-fourth or 13 of the progenies died early without apparent segregation, checking with bench No. 1. The remaining 35 appeared to be segregating for various degrees of resistance.

In summation, it is evident that definite genetic segregation of factors for resistance is taking place in the later generations of two crosses tested. The number and relationship of the factors involved remains to be proved. Present results indicate that at least two major dominant complementary genes with perhaps a third of minor effect could be involved. The studies are being continued and the above results will be checked by a more thorough test of progenies from a larger number of selfed plants of both F_2 and backcross generations.

SUMMARY

1. Tests were made with 56 inbred lines and certain single crosses of maize to determine the relative resistance to bacterial wilt as caused by artificial wound inoculations with *Bacterium stewartii* (Smith) Migula.

2. Symptoms and types of reaction varied with the host line. In several of the susceptible lines infection initiated a modification in the development of the vascular bundle. The parenchyma cells around the plugged protoxylem are replaced by heavily lignified cells radiating out in all directions. These show indications of being transformed into conducting elements. This condition was not found in the most susceptible line W-134 and may be a partial explanation as to why it wilts more readily soon after inoculation.

3. All gradations from highly resistant to highly susceptible lines were found. The majority of the field corn inbreds were resistant; the majority of the inbreds of the Evergreen group were intermediate; and the majority of the early sweet corn inbreds were susceptible.

4. Dominance of resistance was found in all F_1 material tested. In a few cases the F_1's were more resistant than either of the parents.

5. Results from the backcross and later-generation progenies of the crosses OSF X WF and OSF X W-134 show definite segregation of factors for resistance with a strong indication that two major dominant complementary genes with perhaps a third, modifying gene were involved.

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PLATE I

A. Normal vascular bundle. Note the thin walled parenchyma cells adjoining the annular or spiral vessels of the protoxylem. GB 799 (200 x)

B. Infected vascular bundle showing abnormal condition of the protoxylem. The thin walled cells have been replaced by heavily lignified cells. Proto-xylem and meta-xylem completely plugged. GB 799 (200 x)

C. Higher magnification of B (400 x)

D. Infected bundle showing bacteria in one of the tracheal tubes (700 x). GB 799

PLATE I
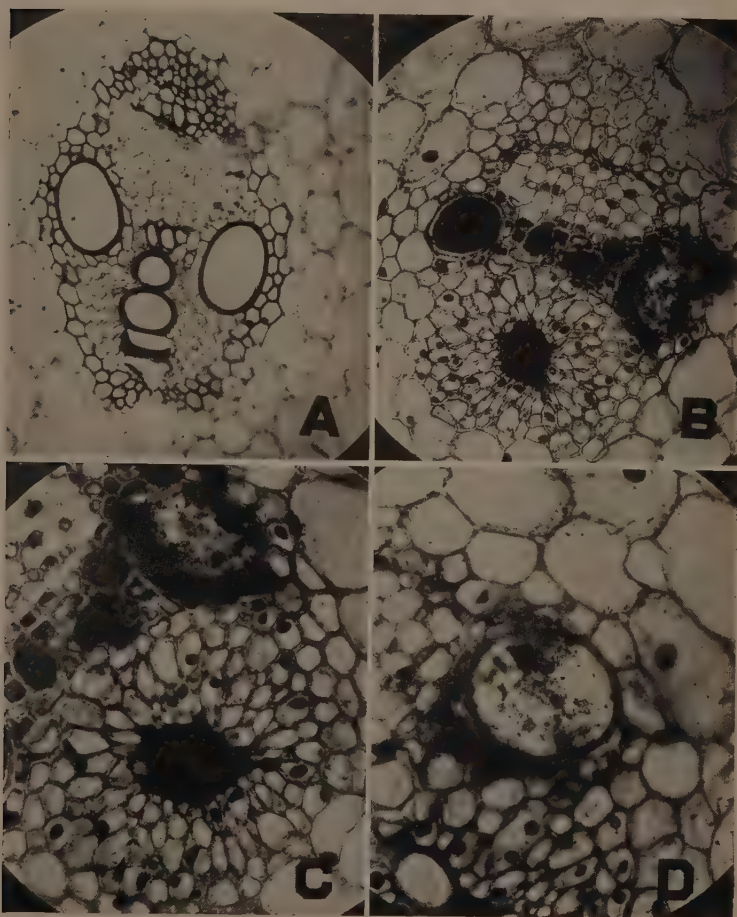
CHROMOSOME STUDIES IN BLACK MEXICAN MAIZE I.

BEHAVIOR OF EXTRA CHROMOSOMES IN BLACK MEXICAN INBREDS AND HYBRIDS WITH DENT TYPES OF MAIZE

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Many cytological studies of maize have demonstrated that 20 chromosomes are characteristic of this species. Exceptions have been noted, especially in Black Mexican sweet corn, in which extra chromosomes are nearly always found. This variety has been studied and extra chromosomes noted by Kuwada (1915, 1925), Reeves (1025), Fisk (1925) and Randolph (1927).

The material for the present investigation, consisting of inbred lines of Black Mexican and F_1 crosses with dent corn, is a part of Dr. Lindstrom's genetic maize material at Iowa State College. The Black Mexican lines have been inbred for eight years, and should be relatively homozygous. Such selection as has been practiced in maintaining these lines has been for typical Black Mexican characteristics only, and no attention has been given to chromosome number. A casual investigation of some of the lines during the summer of 1933 revealed the presence of extra chromosomes. Certain questions then arose concerning the cytology of the Black Mexican lines: (1) were there extra chromosomes in all of this material; (2) if so, were the numbers constant within lines; (3) were the extra chromosomes fragments or whole chromosomes; (4) was their meiotic behavior normal or otherwise; and (4) what is the possible evolutionary significance of the extra chromosomes maintained in established inbreds?

MATERIALS AND METHODS

Eleven inbred or selfed lines of Black Mexican maize and eight F_1 hybrids between certain of these lines and nine-year-old inbreds (selfed lines) of dent maize were selected for the study. Root tips were used for an investigation of the somatic chromosomes of the various lines and hybrids. These root tips were not taken from the same plants on which a meiotic study was made. The root tip sections were stained with iodine-violet and with iron hematoxylin. Material for the meiotic study was grown in the greenhouse. Anthers from young tassels were killed in alcohol-acetic fluid as recommended by McClintock (1929b). The staining procedure was essentially that recommended by her for permanent acetocarmine smears with a few changes. Instead of securing the cover slip with gum mastic to prevent drying, the satisfactory slides were placed in a moist chamber for about an hour, or until the stain had become sufficiently intense. The cover slip was then soaked off in 10 per cent acetic acid. Only rarely does material adhere to the slide itself. Therefore, only the cover glass was run through the following fluids: 30 per cent alcohol to 70 per cent acetic acid, 90 per cent alcohol to 10 per cent acetic acid, absolute alcohol twice, xylol, and finally mounted in balsam. Very satisfactory permanent slides may be quickly prepared by this procedure.

EXPERIMENTAL RESULTS

SOMATIC CHROMOSOMES

In table 1 are listed the results of the study, and as may be observed, extra chromosomes were present in the root tips of all except one cross, No. 13 in the table. All the Black Mexican lines had extra chromosomes, the number of extras ranging from 2 to 10. The number 24 was found in eight of the Black Mexican lines (fig. 2) and in four crosses. In one Black Mexican line and in three crosses there were 22 chromosomes (figs. 1 and 7) and in one cross there were only 20 (fig. 6). One Black Mexican line had 26 chromosomes, and one varied from 20 to 30, the latter being the commoner number (figs. 3 to 5). There is nothing, either in size, shape or behavior of the extra chromosones to indicate that they might be merely fragments. Behavior at anaphase is entirely normal. There is no lagging or other irregularity. This suggests the presence of spindle fiber attachments in all the extra chromosomes.

TABLE 1. *Chromosome numbers in the Black Mexican lines and crosses*

Lab. no.	Culture no.	Material	2n	n
1	S796	Black Mexican	22	11_{II}
2	S1857–1	,, ,,	24	$10_{II} + 1_I$
3	S790–1	,, ,,	24	12_{II}
4	S789–1	,, ,,	24	14_{II}
5	S787–1	,, ,,	24	12_{II}
6	S1855–1	,, ,,	24	12_{II} and 13_{II}
7	S1864–1	,, ,,	24	12_{II}
8	S1861–1	,, ,,	24	12_{II}
9	S1429–3	,, ,,	24	$11_{II} + 1_I$
10	S1866–1	,, ,,	26	13_{II}
11	S1427–1	,, ,,	26, 28, 30	....
12	S1857–2 X3399	B.M. x Bl*	22	11_{II}
13	S1856–1 x 9423–51	B.M. x rdg	20	10_{II}
14	S790–4 x 1807–6	B.M. x — —	24	12_{II}
15	S789–3 x 1806–3	B.M. x Sc	24	11_{II}
16	S787–3 x 1742	B.M. x Bls	24	12_{II}
17	S784 x 1697–5	B.M. x La	22	10_{II}
18	S791 x 1724	B.M. x Ldg	24	....
19	S784 x 1795	B.M. x Wal	22	11_{II}

* These lines, Bl, rdg, etc., are inbred lines of dent corn and constitute a part of Dr. Lindstrom's genetic maize material.

MEIOTIC CHROMOSOMES

In normal maize at early diplotene or very late pachytene, there are many points at which opening-out is starting. The chromosomes begin to contract and continue to do so uniformly until metaphase when they are nearly spherical. Both divisions are then regular.

In cells containing extra chromosomes, behavior may be regular, but frequently it is not. There is also a distinct difference in behavior between bivalent and univalent extras. In middle diplotene, when the chromosomes are still relatively long and contain fairly numerous chias-

mata, a univalent chromosome may be recognized (Pl. I, fig. 5) by the fact that it has already contracted until it is in a stage approximately equal to late diakenesis. In diakenesis it may also be recognized by its greater contraction and because it is smaller and shows no evidence of doubleness.

At metaphase the univalent is frequently not oriented and, whether this be true or not, it invariably proceeds to the poles ahead of the other chromosomes (Pl. I, fig. 6). There was nothing in any of the cultures with one univalent chromosome to indicate that it was a fragment. In size it resembled the other chromosomes after disjunction (fig. 18).

The meiotic number in the Black Mexican lines varied from $10_{II}+1_I$ to 14_{II} (figs. 9, 13, 14), and in the F_1 from 10_{II} to 12_{II} (figs. 10 to 12). In the crosses the extra chromosomes were always present in pairs. Behavior of the extra chromosomes is essentially the same in both the Black Mexican lines and in the crosses.

The extra chromosomes, when paired, could not be distinguished from the others until early diakinesis (fig. 16) and not always then. At that stage there is frequently evidence of a weakness in the pairing union between the members of the bivalent. This often leads to the complete separation of the bivalent into univalents. This tendency becomes more marked as metaphase is approached (fig. 14). If such abnormal behavior is not present the extra chromosomes cannot be detected until later.

There are several departures from the normal behavior which may be observed at metaphase and anaphase. Whether the extra chromosomes reach the metaphase condition as bivalents or as univalents they may be correctly oriented on the plate, or they may lie between the plate and one of the poles. Correct orientation is usually found but non-orientation is by no means rare (fig. 15). If the bivalent comes to the plate, it will disjoin normally with the slight exception that it usually disjoins before the other chromosomes and its members frequently have reached the poles by the time the other chromosomes are disjoining (Pl. I, fig. 4). If the extra bivalent is not oriented, there is evidence that disjunction is at least attempted (fig. 15). There are attenuations toward both poles indicating a spindle pull. There is no evidence of what finally occurs except that eventually the chromosomes are included in one or both polar groups. Micronuclei were not observed.

Should the bivalent reach metaphase as univalents, the subsequent behavior is that characteristic of univalent chromosomes. They proceed at random to the poles, one to each pole, or both to one pole, the latter being the equivalent of non-disjunction. When non-orientation occurs both chromosomes seem to be included at the pole nearest to which they lie. It should be remembered that regardless of the type of behavior, whether one or all types are found in a single cell there are never micronuclei and all the chromosomes become included in one or the other polar group.

Behavior at second metaphase is essentially regular. There is occasionally some lagging, but not enough to prevent the chromosomes from reaching their poles. Only in culture No. 19, a cross, were there countable figures at second metaphase. These showed two combinations of numbers, 11 and 11; and 10 and 12. The 11 and 11 combinations were the more frequent (figs. 20 and 21).
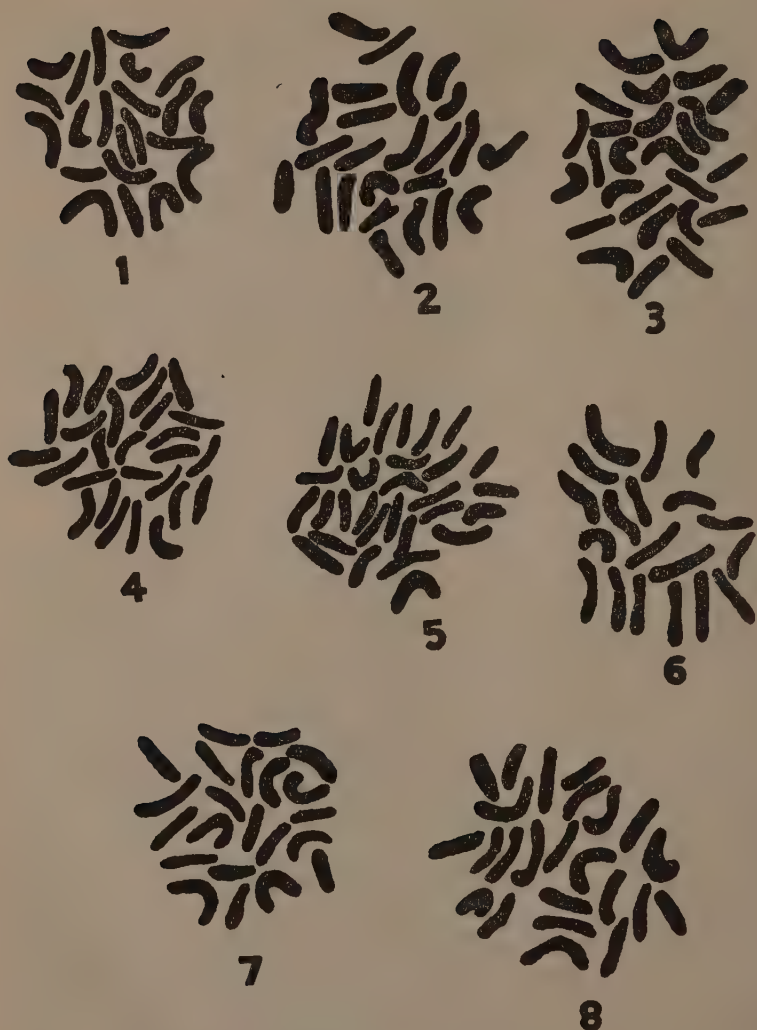
Figs. 1-8 x 1600. Metaphase chromosomes from root tips.

    1. Culture 1, 22 chromosomes; 2. Culture 5, 24 chromosomes; 3. Culture 10, 26 chromosomes; 4. Culture 11, 28 chromosomes; 5. Culture 11, 30 chromosomes; 6. Culture 13, 20 chromosomes; 7. Culture 12, 22 chromosomes; 8. Culture 16, 24 chromosomes.
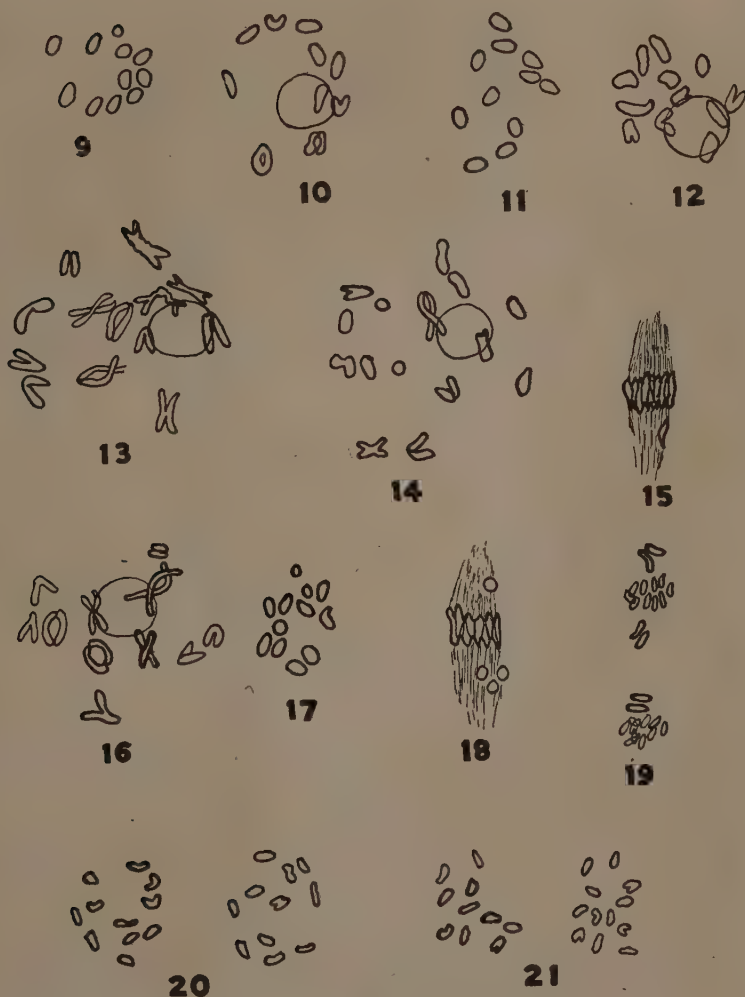
Figs. 9-21. Chromosomes in meiosis. x 1600.

9. Culture 2, $10_{II} + 1_I$; 10. Culture 17, 10_{II}; 11. Culture 15, 11_{II}; 12. Culture 14, 12_{II}; 13. Culture 10, 13_{II}; 14. Culture 4, $13_{II} + 2_I$; 15. Culture 15, one non-oriented pair; 16. Culture 19, diplotene showing extra pair much contracted; 17. Culture 12, $10_{II} + 2_I$; 18. Culture 3, unequal separation of four univalents; 19. Culture 10, unequal distribution of 3 pairs; 20. Culture 19, M_{II} even distribution 11 and 11; 21. Culture 19, M_{II} uneven distribution 10 and 12.

DISCUSSION

It is evident from the data that the Black Mexican lines carrying extra chromosomes give rise to gametes with varying numbers of extra chromosomes. The variability within lines indicates this, as does such a cross as No. 16 with 12 pairs of chromosomes, whose Black Mexican parent came from a line containing 12 pairs. The same is true of the cross, No. 14, and its Black Mexican parent. In the cross, No. 18, there were 24 chromosomes in the root tips, and the same number in the root tips of the line from which the Black Mexican parent came. In the meiotic material the cross had only 11 pairs, while the Black Mexican line had 14 pairs. In the crosses, Nos. 13 and 15, both with the same Black Mexican strain, the somatic numbers were the same, but the meiotic numbers were 10 and 11 pairs.

If the meiotic behavior were entirely regular and the extra chromosomes behaved as the others, these irregularities in number would not occur. However, the study of the meiotic material showed that behavior is not regular and it also showed the cause of the variation in number. The fact that the extra chromosomes sometimes enter metaphase as univalents and thereafter behave as such is the explanation. Thus in a plant containing 22 chromosomes, gametes with 10, 11, and 12 chromosomes may be produced. The greater the number of extra chromosomes the more variability may be expected.

The fact has already been demonstrated (McClintock, 1929a) that extra chromosomes may be carried in the spores of maize without much resultant sterility. The pollen of Black Mexican appears entirely normal, apparently suffering no harm from the presence of extra chromosomes. It seems fairly obvious that there is no strong selective force against the presence of extra chromosomes in this variety of maize or they would have been eliminated before nine generations of inbreeding. On the contrary, it would seem that there must have been a selection favoring the extra chromosomes or some of the lines would have retained the normal number. Perhaps the extra chromosomes are in some way associated with certain characteristics peculiar to Black Mexican, and that while selecting for these phenotypic characters, one unconsciously selects for the higher chromosome number as well.

The extra chromosomes do not seem to be fragments. In the somatic material there was nothing, either in their size or behavior, to indicate such origin. They obviously have spindle fiber attachments, and except for their somewhat erratic behavior during meiosis, are evidently good chromosomes. The question of their origin is one that can be answered by speculation only. It seems unlikely that they could have arisen by fragmentation for the extra parts would have been lost without spindle attachments unless there could have arisen *de novo*, and there is no evidence that such a phenomenon occurs.

A logical explanation seems to be that non-disjunction gave rise to gametes with extra chromosomes, thus eventually producing plants with extra pairs. There is an objection to this argument, also. If the extra chromosomes are duplicates of some of the others there should be formed some tetravalents at meiosis, but there was no indication that this occurred. If Black Mexican sweet corn, as a distinct variety of maize, were old enough that the composition of the extra chromosomes could have

been so changed by evolution (mutation particularly) that they were no longer homologous with any of the others, the non-disjunction explanation might seem reasonable. But Black Mexican, as a variety, is of fairly recent origin. It is, of course, possible that the extra chromosomes were present in maize long before this particular variety was developed.

Regardless of our lack of knowledge of the content, function, and origin of these extra chromosomes, their presence is of possible evolutionary significance. The mere fact that they can be carried on without detriment to the species is significant. Their behavior may be a means of increasing the chromosome number where it is not increased by the usual method, polyploidy. By the Black Mexican method such increases as are seen in Carex, Crepis, and even Drosophila could be accounted for. As has been seen in Black Mexican sweet corn, this variability has caused numbers from 22 to 30, and if during evolutionary time some of these numbers became stabilized it is easy to see the beginnings of a new constant chromosome number, either one or several pairs higher than the original.

SUMMARY

The somatic and metiotic chromosomes of eleven inbred lines of Black Mexican sweet corn and eight F_1 crosses with inbred dent types were studied.

Extra chromosomes were found in all the Black Mexican lines and in seven of the crosses.

Gametes wtih varying chromosome numbers arise in Black Mexican.

Variability of number was found within lines, and numbers higher than those expected were found in the crosses.

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EXPLANATION OF PLATE I

Fig. 1. M$_I$ of culture 15 showing 11 pairs of chromosomes x 900.

Fig. 2. Diakinesis of culture 15 showing 11 pairs of chromosomes x 900.

Fig. 3. Diakinesis of culture 10 showing 12 pairs of two univalents x 540.

Fig. 4. M$_I$ of culture 15, precocious division of the extra pair x 900.

Fig. 5. Diplotene of culture 2, univalent much contracted.

Fig. 6. M$_I$ of culture 9, precocious advance of univalent.

Fig. 7. Anaphase I of culture 15 showing precocious separation with non-disjunction and lagging.

PLATE I

1

2

3

4

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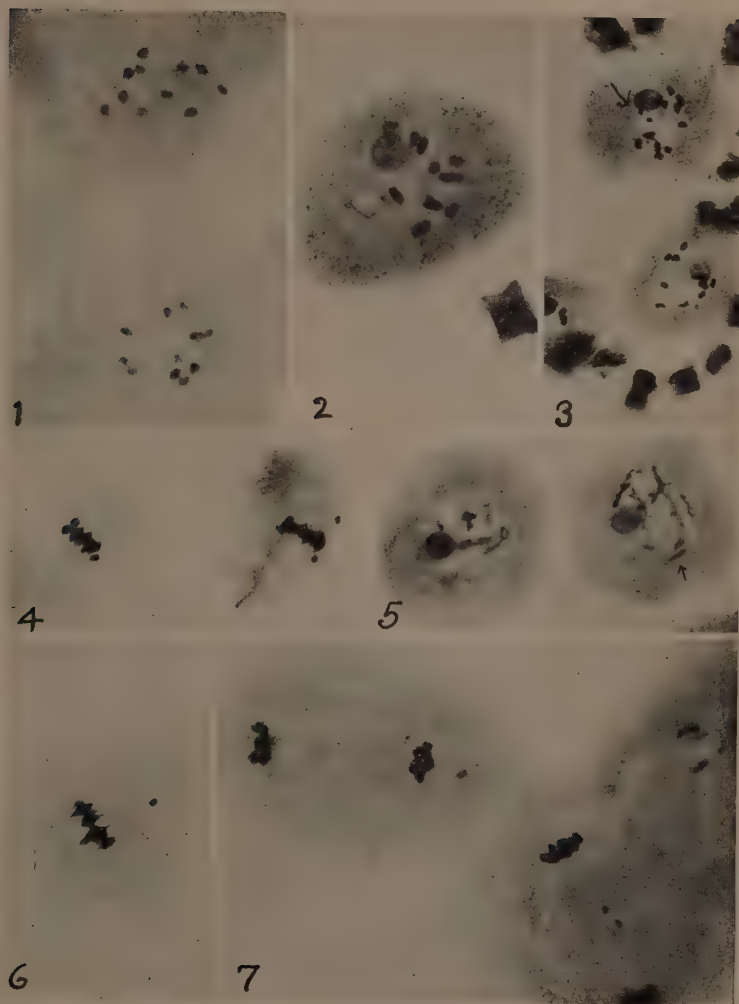
THE FUTURE OF CORN PRODUCTION

H. D. HUGHES

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Not long since, to give careful heed to past developments in their relation to the present was considered a wise means of anticipating events and conditions of the immediate, and more projected future. But the cataclysmic development during the past few years and months have vitiated this method until today who dares predict what the morrow may bring forth.

The Secretary of Agriculture has frequently stressed, very earnestly and prayerfully I know, his conviction that if, as a nation, we are to find our way into that finer, more satisfying, and satisfactory way of living there must come a change in our very innermost lives, and that with that change will come a different viewpoint and a new approach to problems, economic and social. Perhaps for this more time will be required than for physical and material changes, but let us hope not so long a time, that the very opportunity is gone never to return.

It seems most fitting, in connection with the first public recognition this day of the Corn Institute, that the Secretary of Agriculture should participate and lend the prestige of his high office.

Also, it is especially fitting that the Iowa citizen, H. A. Wallace, should have special recognition in this week's program because of the very important contribution which he personally has made to our knowledge of the corn crop and its improvement. Not only has he placed himself in the forefront of the experimentation, personally, but has been equally successful in stimulating others to make worthwhile contributions to our knowledge of corn.

The conception, by the Director of the Agricultural Experiment Station, of a Corn Institute to be sponsored jointly by the Iowa Agricultural Experiment Station and the United States Department of Agriculture, to be located here where the corn crop has always been recognized as of paramount importance, not only to those who produce it but also to every person in the state no matter what his business or calling, was recognized instantly as altogether desirable. Thus, there has developed this research corn program wherein the production and improvement, the manifold uses of the product itself, and its marketing shall receive the minute and the meticulous study which its importance and the exigencies of the times appear to warrant.

Agriculture rightfully expects of the state agricultural colleges and experiment stations important contributions to our knowledge of efficient corn production, to the enlargement of its uses, and to the creation of new industries which will utilize its potentialities. This, we believe these institutions have been doing in the past, attempting to solve the problems of the day and to anticipate somewhat those of the future.

In the face of a surplus production problems of such magnitude as to require for the national welfare the evolution of a control program,

limiting and reducing production, it must seem astounding to those who have not given special thought to the subject that this college and the federal government should go on record advocating the general adoption of more efficient production methods.

It is obvious, however, that to follow any other program must result in an increase in the cost of producing a bushel of corn, or a hundred pounds of pork, to such an extent that, even though the price to the consumer be increased, there is no resulting increase in the purchasing power of the farmer. The basis for the plan evolved whereby the federal government has supervised a program limiting and reducing production, is the advantage to the industrial workers and urban citizens generally of a relatively high purchasing power for the farm group.

The Iowa State Corn Yield Test was inaugurated in 1920, largely due to Mr. Wallace's vision and foresight, with the definite objective of finding the best strains and varieties of corn for planting in the different parts of the state—those most efficient in using soil nutrients, sunshine and water, converting them into grain and forage—those which when planted in comparison with other varieties may be expected to give the larger acre yields. To increase the total production of corn in the state was not the goal, but that each bushel might be produced at a lower cost.

With the general use of improved practices and soil management methods the Iowa corn acreage could be reduced a third and the total production of corn greatly reduced without a reduction in net returns to the corn growers; or that with such a change the soil fertility could be maintained, soil losses from erosion reduced to a minimum and production cost per bushel lowered, while at the same time, with a stabilized price, the purchasing power of the producer would be materially increased.

Corn growers must expect, and must be expected, to give as much attention to the best and most efficient methods of production in the future as at any time in the past. We believe that the statement of the Secretary of Agriculture, made in the fall of 1933, in which he said that "the federal policy is to promote increased efficiency but on a controlled acreage" must be recognized as sound and in the best interest of all. And thus the research program continues; better corn, better cultural practices, better mechanical equipment and methods, all looking to more efficient production—lower costs of production.

SIX DECADES OF CORN IMPROVEMENT AND THE FUTURE OUTLOOK

H. A. WALLACE

United States Secretary of Agriculture

Of all the annual crops, corn is one of the most efficient in transforming sun energy, soil fertility and man labor into a maximum of food suitable for animals and human beings. Corn more than any other one plant is the foundation of the civilization of the great democratic Middlewest. The livestock and the rotations which properly go with corn growing make for a continuing democracy. It is, therefore, appropriate that the people of the corn belt, whether they live on the farm, in the small towns or the great cities, should have a genuine sympathy for corn and a deep understanding of it. It is to be regretted that so few of the millions of people whose prosperity rests on the corn plant should have so little appreciation or knowledge of it. Even those who work most with corn display little of the genuine reverence for it which characterized the majority of the corn growing Indians up until the 20th century.

It has been suggested by Dr. Melhus that I should discuss this evening "Six Decades of Corn Breeding and the Future Outlook." The presentation which I shall make this evening will, of necessity, be informal and neither detailed nor scientific. It happens that during the past two years I have had little opportunity of following with my former interest the developments of scientific corn breeding. With this qualification, it is a great pleasure to present for your consideration the following rather brief summary indicating my own particular attitude concerning the corn breeding methods of the past and the immediate future.

The past sixty years may be roughly divided into four main periods. During the first period from 1874 to 1893, corn breeding was chiefly in the hands of practical farmers. During the second period, from 1893 to 1910, the colleges and experiment stations assumed more and more leadership through the mechanism of corn shows and certain rather simple experiments. In the third period, from 1910 to 1920, there was an increasing interest, stimulated largely by the experiment stations in ear-row breeding, crossing of varieties and variety yield testing. Only in the fourth period, since 1920, has the problem of corn breeding been approached in a comprehensive, scientific way.

In each case there was, of course, a transition period that has arbitrarily been thrown into the earlier period. Then too, many exceptions may be taken to this rough division. Beal of Michigan, for example, crossed corn varieties to increase yield in the late seventies. There were a few corn shows in the eighties and ear-row corn breeding began at the Illinois Station in the late nineties. Hartley of the U. S. Department of Agriculture did a little inbreeding of corn about 1900. But in spite of these and other apparent exceptions, it may be said that in the main the last six decades divide broadly as I have indicated.

Previous to 1920 the methods of corn breeding in practical use were for the most part no great advance over the methods in use by the Indians. It is true that the Indians, being interested primarily in corns they could grind for human use, favored flour types which do not yield as well as the dent corn commonly grown by the white man in the corn belt. The Indians of the southern corn belt maintained a certain amount of dent corn but it did not have as wide a use for their purpose as the earlier flour and flint varieties. The dent sorts which the white man took over from the Indians of the southern corn belt were decidedly variable. They probably were crosses of flint corn with gourd seed or shoe peg which carried twenty-four rows or more of very deep, narrow kernels of soft texture, deeply dented. The gourd seed corn which today has practically passed out of existence in its pure form probably yielded fairly well on the bottom lands of the Ohio, Tennessee and lower Mississippi and Missouri Rivers. The stalk was evidently large but it was also probably subject to diplodia, fusarium and most of the other corn diseases.

Robert Reid, who moved from southern Ohio to central Illinois in 1846, was one of the first white men, whose work has definitely endured to this day, to select a definite dent type from a cross of what apparently was a late semi-gourd seed of southern Ohio with an early sort known as the "little yellow". The phrase "little yellow" was customarily used previous to the Civil War to designate early flint corn. Robert Reid in moving from Ohio to Illinois took his late Ohio corn with him and the germination was so poor that he replanted the missing hills with what was probably an early yellow flint. The Ohio corn is said to have been reddish in color and the problem before Robert Reid and his son, James Reid, was to reduce the cross to a certain amount of uniformity. James Reid had the soul of an artist and he set about it to produce a beautiful straight-rowed type of corn with an ear almost as large as the late Ohio parent but with a smooth dent, easy to husk. Year after year James Reid bred for beauty and in 1893 his efforts were rewarded by a first prize at the Chicago World's Fair.

A considerable quantity of Reid Yellow Dent corn was spread over the corn belt under the name of World's Fair corn but the really great spread did not come until about the year 1900 as a result of the evangelical corn show extension methods of Perry G. Holden in Illinois and Iowa. The corn show people during the period from 1900 to 1910 set standards which made Reid Yellow Dent into a rough corn.

Probably there were 50 or more farmer corn breeders in the corn belt who began their work either just before or soon after the Civil War. Among these are J. S. Leaming of Ohio, who originated Leaming corn; James Riley of Indiana, who originated Boone County White; H. J. Goddard of Fort Atkinson, Iowa, who originated Silver King and R. Hogue of Nebraska who originated Hogue Yellow Dent. These various men endeavored to take the mixed corn of the period and make it uniform for color and ear type. They knew little about yield tests but they had their own ideas as to characteristics which might make for yield. Most of these early corn breeders seem to have been sincere lovers of the corn plant and it is appropriate in passing to pay respect to their memory. Most of the corn with which the more scientific corn breeders of today are working is descended from an ancestry which was in the hands of these men.

The corn show period from 1893 to 1910 was of extraordinary interest from a psychological point of view. It was during this period that the farmers of the central corn belt became truly corn conscious for the first time. During this period Perry G. Holden, speaking at the short courses at Ames and elsewhere, inspired literally thousands of farmer boys to study an ear of corn as their fathers had never studied corn. Iowa, Illinois, Indiana and Missouri may have gone during this period to rather foolish extremes in their emphasis on corn shows but the human interest aroused at this time was to prove a powerful dynamo out of which more constructive movements were to develop later.

In the third period, beginning in 1910, there was an ever increasing interest in yield testing and ear-row breeding. Holden had been interested in both of these methods of improvement but his job was of a nature which prevented him from doing very careful work in this direction. The many yield tests which he conducted on the County Poor Farms were designed to prove primarily that the best corn for a farmer to grow was that produced on his own home farm.

The most significant breeding and testing work done during this period was being done by the Illinois and Nebraska experiment stations. Hopkins and Smith started in 1896 their famous chemical selections of corn and developed their theories of ear-row breeding. Montgomery and Kiesselbach at the Nebraska Experiment Station began shortly previous to 1910 a great variety of breeding experiments most of which were later on to be proved futile. The great range in the temperature, altitude and rainfall in Nebraska, however, resulted in Nebraska experimenters developing more forcibly perhaps than anyone else the great need for adaptability.

Most of the corn belt stations during the second decade of the 20th Century conducted enough in the way of yield tests to prove the adaptability of certain strains and varieties for the different parts of their respective states. The work on the whole was somewhat loosely done because there was not sufficient appreciation of the great variability of strains within a variety.

Some of the most thought provoking work during this period was by Williams of the Ohio Station which indicated that smooth corn yielded slightly better than rough corn and that most of the ear characteristics had very little influence one way or the other. Williams of Ohio and Kiesselbach of Nebraska probably did as much as anyone during the second decade of the 20th Century to shake corn breeders out of their previous complacency. To some extent they were both destructive critics because they did not point the way very definitely as to what should take the place of the corn show.

The first real progress in comprehensive scientific corn breeding began about 1920. It was in the early 1920's that the scientific yield testing of M. L. Mosher of Woodford County, Illinois, demonstrated that Krug corn was definitely superior in yielding power to the showier looking strains of Reid Yellow Dent. It was in 1920 that Professor H. D. Hughes started the Iowa Yield Test which was destined to continue longer and on a more scientific basis than any other yield test which has thus far been conducted anywhere at any time. In 1920, F. D. Richey of the United States Department of Agriculture, began his significant work

in coordinating the corn breeding experiments of the different experiment stations in a more definite national program. About 1920 Donald F. Jones of the Connecticut Experiment Station began to publicize the possible commercial significance of crossing four inbred strains of corn in what is called a double cross.

The rapid increase in genetic knowledge following the rediscovery of Mendel's results in 1900 had established a firmer basis for more scientific corn breeding. The genetic researches with maize by East and Hayes, by Emerson, by Collins and by others all were contributing their part. George Harrison Shull of the Carnegie Institution and Edward Murray East of the Connecticut Experiment Station had conducted their epoch-making experiments with inbreeding and crossing inbred strains of corn during the period from 1905 to 1911. These classical foundation experiments had practically no influence, however, on corn belt experiment stations until the period beginning with 1920. At that time Donald Jones of the Connecticut Station, H. K. Hayes of the Minnesota Station, F. D. Richey of the U. S. Department of Agriculture, and James R. Holbert also of the U. S. Department of Agriculture, and several others served as centers of a growing enthusiasm for the development of superior inbred strains of corn and the developing of methods for the practical utilization of such strains. One by one the different experiment stations started comprehensive programs in the developing of inbred strains of corn.

In Minnesota, H. K. Hayes, who had been associated with E. M. East in his fundamental work at the Connecticut Experiment Station from 1908 to 1914, was developing early inbred strains from Minnesota 13, Rustler and Northwestern Dent. The Iowa Station at Ames started its ambitious program in the spring of 1922 under the direction of Merle T. Jenkins, who continued to direct it until 1934 when he was placed in charge of the corn investigations in the U. S. Department of Agriculture and A. A. Bryan took over the Iowa program. James R. Holbert, working on corn diseases for the United States Department of Agriculture, had started his inbreeding work from a disease resistance standpoint in Illinois a few years previously. About 1918 Hoffer at the Indiana Experiment Station at Purdue started his excellent work, now being continued by St. John and Trost and Smith.

Brunson at the Kansas Station, Kiesselbach of the Nebraska, Stadler of the Missouri Station, a group at the Wisconsin Station and Meyers and Stringfield in Ohio, all began careful corn-inbreeding programs in more or less close cooperation with the United States Department of Agriculture. Beginning about 1925, it was demonstrated on a large enough scale to indicate practical certainty that the crosses of inbred strains were definitely superior in yielding power to the regular open-pollinated varieties. The first combinations of inbred strains were really not so very good but to the experienced eyes of men close to corn, the eventual possibilities seemed extraordinary. As the years went by, the combinations were more and more improved and efforts were made to combine high yields with stiffness of stalk, resistance to disease and resistance to drought.

While this fascinating but somewhat empirical work in the development of inbred strains of corn was going on and discoveries were being made as to which inbred strains of corn would combine to the best advan-

tage, the geneticists such as R. A. Emerson of Cornell University, E. W. Lindstrom of Ames, Brink of Wisconsin, Stadler of Missouri, and Collins and Kempton of the U. S. Department of Agriculture were delving into the fundamentals of the corn chromosome map. Emerson, a pioneer in this work, acted as a coordinator and stimulated other investigators so that much more rapid progress was made toward a complete map. Many hundreds of Mendelian allelomorphs have been discovered and a great many of them have been placed on the proper chromosome. Something is known as to the exact position of many of these allelomorphs and observations have been made as to the frequency of crossing over. Thus far, very little practical utilization has been made by the corn breeders of the detailed knowledge possessed by the geneticists. It is conceivable, however, that in the near future more experiments will be set up to discover the significance from a yield and disease resistant standpoint of the dominant or recessive aspects of specific allelomorphs.

Some 10 years ago Richey started an experiment to determine the relative effect on yield of the dominant and recessive factors for plant and aleurone color. I was sorry that the stocks were lost by flood as I had been much interested in it. However, some recent preliminary work by Brink of the University of Wisconsin indicates that corn with a genetic composition of A-b-Pl may yield definitely more than corn with a genetic composition of A-B-Pl or corn with a genetic composition of A-b-pl. Corn with a genetic composition of A-B-pl seems to have a like advantage. In other words, preliminary results indicate that perhaps corn with the plant color known as dilute purple or sun red may have an advantage over ordinary corn or over corn with a plant color of true purple. Brink does not look on his experiments as at all conclusive as yet, but they are suggestive of the desirability of setting up in a careful way over a period of years experiments designed to discover more definitely the true functional significance in varying combinations of different allelomorphs which thus far have been known chiefly by their superficial external manifestations. Such experiments would provide a good opportunity for joint attack by the geneticists and the breeders. While a joint attack of this sort would seem to me to be of eventual profound significance, I am inclined to think that during the next ten years, the best combinations will be discovered by cut and try methods of the intelligent and industrious breeder who makes large numbers of combinations and observes them carefully under a variety of conditions. The combination of the genetic and breeding approach to discover the physiologic effect of various genes in different relationships will, I feel, lead finally to exceedingly promising results but no one can predict at the present time just what turn research of this sort will eventually take. I am convinced, however, that after work of this sort is started, it will lead to a number of fresh and worthwhile approaches within five or ten years not only so far as the corn plant is concerned, but also with respect to other organisms.

The methods of producing and crossing inbred strains of corn as used in 1934 will probably be greatly modified as the combined attack of the geneticists and breeders brings us closer to the truth of genetic and physiologic functioning. I can't help feeling that Mendelism is being used to explain situations for which eventually other hypotheses must be used. In this connection the effort to develop superior inbreds by the

method known as convergent improvement is worth studying. The method is slow and thus far has not produced results quite as favorable as might be hoped. My own observation would lead me to think that superior inbreds are superior not only from the standpoint of the presence of specific favorable Mendelian factors but also from the standpoint of a certain degree of tension resulting from compatible relationships and arrangements, the laws of which have not yet been discovered. In this direction I am expecting profoundly significant work in corn genetics in the next 20 years which will have its eventual application to all life.

Looking toward the future, it is obvious that the surface of the possibilities in corn breeding has merely been scratched. It is undoubtedly true that the use of the combinations of the inbred strains of corn which are now clearly within our grasp should make it possible for the farmers of the United States within the next ten years to obtain a yield at least five or ten bushels an acre higher than that which they are now obtaining. It is also obvious that stiffer stalked strains are clearly within our grasp. There is still the problem of obtaining in one combination the qualities of maximum yield, stiffness of stalk, drought resistance and disease resistance, as well as the ability to maintain the same advantage in yield over open-pollinated corn on poor land and rich land. It would seem, however, from the rapidity of progress since 1920 that very remarkable results will be with us by 1940. We shall need experiments to discover whether or not it is desirable to have softer textured strains of corn in order to promote greater ease of mastication by hogs, or whether such problematical advantage in softness may be offset by lower yields and susceptibility to disease. Is there any advantage in increasing or decreasing the oil content in corn from the standpoint of the maximum utilization by livestock? Perhaps the time is now approaching when the corn breeders should cooperate more and more with those who are concerned chiefly with the use of corn. Possibly we shall come eventually to think of one type of corn as hog corn to be fed on the ear, another type as cattle corn to be fed ground, and still another type as commercial corn especially adapted for human consumption, or starch corn for the wet process of corn manufacture.

Most farmers still look on corn as corn. But those who have worked with corn and studied it in all its intimate details for many years realize that corn is not merely corn. It is a composite of many things and can be molded in many directions. The possibilities with corn are almost as infinite as with humanity itself. The past sixty years are a mere beginning. The future is limitless as long as our desires are keen and our minds open.

THE PARASITES OF SOME LEPIDOPTEROUS STALK BORERS IN IOWA[1]

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In the course of studies on the bionomics of several lepidopterous stalk borers that occur in Iowa, much information concerning their parasites has been obtained. Because of the present widespread interest of entomologists and agriculturists in all phases of the biological control of insect pests, several workers have urged the early publication on the observations of the habits and interrelationships of these parasites.

HOST SPECIES

During the past six years ten species of noctuid and two species of olethreutid stalk borers have been under observation in the field and in the Insectary at Iowa State College. Some of these have been studied at length, whereas others have received only brief attention, but from each species one or more species of parasites have been reared. A few of the borers included in these studies are recognized as important pests of cultivated plants and an interest in their parasites arises naturally. Other species attack only weeds and are of little or no economic importance, yet many of their parasites also are parasites of other insects which are recognized as serious pests. There is, therefore, adequate reason for maintaining a natural interest in these parasites and in their weed-infesting hosts. In some cases these hosts serve as reservoirs for maintaining stocks of the parasites; and in other cases they serve as alternate hosts in which the parasites may pass the winter or develop an additional generation of flies or wasps.

The stalk borers included in this study are: *Achatodes zeae* (Harris), *Luperina stipata* (Morr.), *Macronoctua onusta* Grote, *Papaipema nebris* (Gn.), *P. cataphracta* (Grote), *P. arctivorens* Hampson, *P. purpurifascia* (G. & R.), *P. frigida* Smith, *Oligia fractilinea* (Grote), *Archanara subcarnea* (Kell.), *Epiblema otiosana* (Clemens) and *E. strenuana* (Walker). In a few cases where parasites of the foregoing species have been reared from other insects this fact will be mentioned.

MASICERA SENILIS Meig.

Masicera senilis Meig. (*M. myoidea* R. D. of Coquillett) was by far the commonest, and probably the most valuable of all the parasites reared from *Papaipema nebris,* a borer which appeared to be its principal host.

However, many adults of *M. senilis* were bred also from larvae of *Luperina stipata, Macronoctua onusta, Papaipema purpurifascia, P. cataphracta, P. frigida* and *P. arctivorens,* and a few (at least one or more) were reared from *Achatodes zeae, Archanara subcarnea* and *Epibleme otiosana.* In the literature this species is recorded also from several other hosts, including *Gortyna immanis* Gn., *G. stramentosa* Gn., *Nonagria oblonga* Gr., *Pyrausta nubilalis* and several species of *Papaipema.* Bird (1925a) has observed this tachinid to be a parasite of twenty-two species of allied noctuid borers. Britton (1919), Washburn (1910), Bird (1921), Lowry (1927) and others have found *M. senilis (myoidea)* to be an important parasite of *P. nebris* and Breakey (1931) referred to it as one of the most important parasites of *M. onusta* in Wisconsin.

M. senilis is viviparous and caged specimens were observed to deposit their larvae about the entrances to the borer tunnels and at other points on the plant and on the trash in the bottom of the cage, but none were deposited on exposed borers. It seems probable that in the field the maggots would be placed near the tunnel entrance and that the random deposition in the cages was because the borers had recently crawled over everything. Some of the tiny maggots moved slowly about, whereas others remained comparatively quiet. To some extent they may search for their host, but perhaps most of them first encounter the borer when it is casting refuse from the burrow. Once inside the borer the maggot apparently develops slowly until the host is nearly full-grown and then it rapidly consumes most of the body tissues. The parasite pupates near the remains of its host either in the burrow or in the soil. The length of the pupal stage varied between 10 and 21 days and averaged 14 days. Adult emergence extends over a rather long period of time, beginning July 1 (from *A. zeae* and *L. stipata*) and continuing until September 15 (from *P. nebris* and *M. onusta*). Bird (1916) has found that some of the late formed puparia overwinter and yield adults the following July. The writer has not been able to verify this observation. In his experiments a number of puparia of assorted sizes have been placed in soil out-of-doors each year, but only adults of *Gymnochaeta ruficornis* Will. emerged in the spring. The alternative possibly is that this species, like the European homonym, passes the winter as a larva in some alternate host.

In the majority of cases but one fly emerges from a single host; the emergence of two flies, however, is not uncommon, and, in a few cases, three flies have been reared from a single host. In cases where more than one parasite developed in a caterpillar the resulting adults were usually smaller than the others.

This fly commonly parasitized between 8 and 30 per cent of *Papaipema nebris* larvae and in some cases it attacked as high as 70 per cent of the borers. The degree of parasitism in the other borers was generally somewhat lower. *Masicera* was reared from borers collected in corn and other cultivated plants as well as from those collected in *Ambrosia.* Bird (1921) has pointed out that *P. nebris* larvae in corn and cultivated crops were practically free from parasites, while Vinal and Caffrey (1919) found that *P. nebris* larvae in corn were very highly parasitized by *Masicera,* but that larvae of *Pyrausta nubilalis* tunneling through the same plant or through plants in the same hill were only parasitized to a very small extent. Lowry (1927) collected eleven stalk-borer larvae in a corn field, all

of which were parasitized by this species. Eight of the borers contained two parasites each.

Eupteromalus dubius (Ashm.), *Plesignathus* sp., and *Perilampus hyalinus* Say were reared as hyperparasites from the puparia of *M. senilis*. *E. dubius* was the most abundant of the three and although it destroyed only a small percentage of the *Masicera* puparia it was present in every large field collection each year. Huber et al (1928) report rearing *E. dubius* from *Masicera myoidea* in Ohio. At Ames, this secondary parasite was reared in large numbers from *Pyraustomyia penitalis* (Coq.). On this species it was especially prevalent in the puparia of the overwintering or spring brood.

ERYCIA DECKERI Curran

Erycia deckeri was described by Curran (1929) from a single female specimen reared from a full-grown *P. nebris* larva on August 18, 1928. This species closely resembles *Masicera senilis* Meig. *(E. myoidea* of Curran)*, and both species were reared from the same lot of borers. The writer has many specimen of *Masicera (Erycia)* not yet examined by specialists, some of which may prove to be *deckeri*.

GYMNOCHAETA RUFICORNIS Will.

This large, metallic green tachinid was reared many times from *P. nebris*, twice from *P. cataphracta* and once from *P. arctivorens*. It was not found in any of the other borers and always occurred in larvae taken well up in the stalks. It remains to be seen whether or not this is characteristic of the species. Borers infested with this parasite develop a swollen appearance in the midbody region during the last larval stadium, and a few days later a large maggot emerges from the caterpillar and pupates. Pupation, which occurs during August, takes place in the borer tunnels or in the soil, depending upon the location of the host at the time. In the laboratory this species pupated readily in any type of receptacle. The winter is passed in the pupal stage, and the adult flies emerge during May and June. The earliest emergence recorded was on May 12 and the latest on June 11. On these dates the host larvae are two or three weeks old. These notes, therefore, indicate that this fly has a one generation life cycle well synchronized with that of its host.

WINTHEMIA RUFOPICTA (Bigot)

Until very recently this species was considered a synonym of *W. quadripustulata* (Fab.), and unfortunately most of the records attributable to it are given under the latter name. The author's specimens were originally identified as *W. quadripustulata* and are so reported in previous papers (Decker, 1930, 1931), but, in his revision of the genus *Winthemia*, Reinhard (1931) recognized them (p. 32) as *W. rufopicta*.

Fifty-two specimens reared from *P. nebris* emerged between July 20 and August 15; six from *O. fractilinea* appeared between July 12 and 21; three from *L. stipata* emerged July 1, 6 and 19; and two from *P. cataphracta* emerged July 26 and 29. Most of the field collections of borers did not yield a single *Winthemia* but when it was present between 10 and 20 per-

cent of the larvae bore one or more *Winthemia* eggs. The degree of parasitism would undoubtedly be increased but for the fact that this species deposits eggs usually on the thorax of the host, and the borers are therefore only susceptible to attack during periods of migration.

Several specimens of *W. rufopicta* were reared from cankerworms in July, 1930, by H. M. Harris and the writer.

LIXOPHAGA VARIABILIS (Coq.)

Many specimens of this parasite were reared from mature larvae of *Epiblema otiosana* and *E. strenuana*. Most of the adult flies emerged from overwintering borers during mid-June, but seven adults were reared from borers of the summer generations. These emerged July 27, August 2, 13, 18, 20, 29 and September 1. Three specimens were reared from half-grown *Papaipema nebris* larvae on July 15, 1928, and two flies emerged from pupae found in the burrows of *Pyrausta penitalis* on August 2, 1927.

L. variabilis was reared from *P. cataphracta* by Washburn (1910). It has also been reported as a parasite of several weed infesting insects (Lepidoptera and Coleoptera) and also from such pests as *Laspeyresia molesta* Busck, *Carpocapsa pomonella* and *Pyrausta nubilalis*. The *Epiblema* larvae, therefore, serve as important overwintering reservoirs for this parasite.

MUSCINA STABULANS Fall

The larvae of this common fly are practically omnivorous. According to the literature they are able to develop upon plant or animal tissue either alive or in varying stages of decomposition. Next to *Masicera senilis* this was the most common fly taken. Puparia of *M. stabulans* were found in the feeding burrows of *Achatodes zeae*, *Macronoctua onusta*, *Papaipema nebris*, *P. purpurifascia* and *Epiblema otiosana*. In many cases the larvae probably had been scavengers in the tunnels, but several specimens of this fly were bred from live borers and present, therefore, positive evidence of a parasitis habit. Cole (1931), Breakey (1929) and others have made similar observations and consider *M. stabulans* to be a true parasite.

MUSCINA ASSIMILIS Fall

A few puparia of *M. assimilis* were taken from the burrows of *Papaipema nebris* and *P. purpurifascia,* but no positive evidence of parasitism was obtained. Breakey (1929) reports this species from *Macronoctua onusta*.

SARCOPHAGA HELICIS Towns.

Sarcophaga helicis has been recorded as a parasite of many Lepidoptera and certain other insects. It has also been reported as breeding on dead insects. A few specimens of this fly were reared each year from the larvae of *Papaipema nebris* and *Achatodes zeae*. Most of the flies obtained, however, were bred from host larvae that were dead when collected or were reared from puparia collected in the borer tunnels. Only three specimens were reared from borers that were alive when collected. Breakey (1931) reported this species as a parasite of *Macronoctua onusta*.

SARCOPHAGA CIMBICIS Towns.

The habits of this species were apparently very similar to those of the *S. helicis*. Adults were reared from the larvae of *Papaipema nebris, P. arctivorens, Oligia fractilinea* and *Epiblema otiosana*. In most cases they were considered to be parasites of the borers, but some of the records are incomplete and it is impossible to state the number of actual cases of parasitism. Breakey (1929) bred *S. cimbicis* from *Macronoctua onusta* in Wisconsin.

ECTOPIMORPHA LUPERINAE Cush.

Ectopimorpha luperinae was described by R. A. Cushman (1931) from a series of specimens reared from the four-lined borer *(Luperina stipata* Morr.) at the Iowa Agricultural Experiment Station. To date it has not been reared from any other host, but it seems probable that an overwintering brood must develop on some other species (possibly one of the cutworms?).

These parasites develop singly within the body of the borer. The stage of development of the host at the time it is attacked by the parasite is not known. Many parasites were reared from borers collected a week or ten days before pupation, and a few were bred from caterpillars taken in the penultimate stage, about two weeks earlier. Parasitized larvae and pupae are normal in appearance, and the presence of the parasite is imperceptible until the adult Ichneumon emerges from the host pupa. This occurs about 15 or 20 days after the caterpillar has pupated, which is only 2 or 3 days before the moth normally emerges. Adult emergence occurs over a period of about a month, beginning in mid-July and continuing into the second week of August. In seventeen field collections of host larvae the degree of parasitism by this species varied between 1.2 per cent and 18.1 per cent and averaged 9.1 per cent.

MICROPLITIS GORTYNAE Rly.

The Braconid, originally described by Riley (1881) from nine specimens reared from *Achatodes zeae* in Iowa, was a common enemy of the Noctuid borers. Many adults were reared from *Papaipema nebris, Luperina stipata* and *Achatodes zeae* and smaller numbers from *Papaipema cataphracta, P. frigida* and *P. purpurifascia*.

It is quite evident that *M. gortynae* has but one generation each year. The larvae pass the winter within their characteristic reddish brown, ribbed cocoons. Pupation occurs in the spring, and the adults emerge during late May and June. Soon after mating the females start out in search of the borers. As a rule borers about one-third grown are selected for oviposition, hence in the early part of the season the parasites attack the larvae of *Luperina* and *Achatodes* and later on they seem to prefer the *Papaipema* larvae. The parasitized borers continue feeding and appear normal until the parasite larvae emerge. In *Luperina* and *Achatodes* this occurs in late June or early July, at which time the host is in its last larval instar, while in the slower developing larvae of *Papaipema* the parasites frequently emerge during July and August from larvae in the penultimate stage. The number of parasites emerging from individual hosts varied between 6 and 42 and averaged 24.6. Larvae emerging from *Luper-*

ina almost invariably construct their cocoons in the soil; those emerging from *Achatodes* form cocoons either in the pupal quarters or in the feeding burrow of the host, usually in the former, and those emerging from *Papaipema* spin their cocoons either in the soil or in the feeding burrow of the host. Cocoons found in the feeding burrows of the host commonly formed an encircling band about the remains of the dead host while those found in the soil were bound together in irregular masses.

The findings of the writer are in accord with the published observations of Bird (1927) and Balduf (1929). Bird gives a detailed account of oviposition and Balduf presents a good description of cocoon-spinning.

Eupteromalus viridescens (Walsh) was the only parasite reared from *Microplitis* in Iowa. In New York Bird (1925a) found *M. gortynae* parasitized by *E. viridescens, Hemiteles tenellus* (Say), *Astomaspis fulvipes* Grav., *Gelis microplitidis* Gahan, *Ethelurgus* sp. and *Thysiotorus* sp. Balduf reported rearing *E. viridescens* from cocoons of *M. gortynae* and *Microbracon lutus* in Ohio and Illinois, while Breakey (1930) working with *Achatodes zeae* in Wisconsin found a closely related species, *Eupteromalus dubius,* parasitizing about 30 per cent of the *M. gortynae* pupae.

METEORUS VULGARIS (Cress.)

This moderately small, gregarious braconid was a common parasite of *Luperina stipata,* but was not recorded from any of the other borers. The parasite larvae emerged from mature host larvae during late June and early July, and the number emerging from each individual host varied between 6 and 17 and averaged 12.3. The host usually died about the time the parasite larvae emerged, but in a few cases the borer was still alive the following day. Pupation occurs in compact, oval, buff cocoons which may or may not be found together. The time spent in the cocoon varied between 5 and 12 days. A number of adults that emerged July 9 were placed in a large vial with three host larvae on July 11. Oviposition was not observed, but on July 22 and 23, seven, eleven and fourteen larvae of this parasite emerged from the caterpillars and spun their cocoons. The adults appeared five days later.

Meteorus vulgaris is frequently mentioned as a parasite of various species of cutworms. In Saskatchewan, according to King and Atkinson (1928), this species has at least two generations a year and passes the winter as larvae in hibernating cutworms. From these observations we may assume that in Iowa there are at least three or four generations of this braconid each year.

SAGARITIS OXYLUS (Cress.)

Two specimens of this species were reared. One, on August 10, from a pupa collected with the remains of a nearly mature larvae of *Papaipema nebris* and the other, on July 12, from a larva of *Oligia fractilinea. Sagaritis oxylus* is reported as a parasite of armyworms and cutworms and was previously reared from *P. nebris* by Lowry (1927).

LISSONOTA BRUNNEA (Cress.)

Lissonota brunnea, a large Ichneumon parasite, was reared in considerable numbers from the mature larvae of *Papaipema nebris* and in

smaller numbers from *P. cataphracta, Achatodes zeae* and *Luperina stipata*. The large, white, parasite grubs emerged from full-grown borers and immediately spun elongate-oval, closely woven, brown cocoons in which they passed the winter. Adults emerged during April. During the warm hours of the day these adults were very active, but when the temperature dropped below 50° F. they became rather sluggish, yet they were able to show feeble movements of the legs and antennae at 30° F. It seems probable that there is a spring generation of this *Ichneumon* on some alternate host.

Breakey (1930) found three *Lissonota* sp., probably *brunnea* Cress., cocoons in the pupal chambers of *A. zeae,* which in all cases were parasitized by *Eupteromalus dubius*.

AMBLYTELES JUCUNDUS (Brulle)

Several specimens of this large *Ichneumon* were reared from *Papaipema nebris;* three from *Macronoctua onusta* and two from *Luperina stipata*. In all cases the adult parasites emerged from pupae of the host. Those emerging from *L. stipata* appeared July 26-27, whereas those emerging from the other borers emerged in late August and early September.

Dietz (1928) bred *A. jucundus* from *M. onusta* in Indiana.

AMBLYTELES LAETUS (Brulle)

Three adults of *Amblyteles laetus* emerged from *Papaipema nebris* pupae the first week of September, 1931, and a single adult emerged from a pupa of *Oligia fractilinea* July 28, 1928. Bird (1923) states that *A. laetus* is parasitic on the majority of species (of *Papaipema*) which pupate in their burrows and that the adults which emerge from *Papaipema* hosts during September hibernate and presumably have an alternate host in the early part of the following season. Breakey (1931) found *A. laetus* parasitizing three per cent of the *Macronoctua onusta* larvae under observation in 1929. Washburn (1910) bred this species from *P. nebris* and *P. cataphracta* in Minnesota.

APANTELES PAPAIPEMAE Mues.

Apanteles papaipemae was the most common hymenopterous parasite of *Papaipema nebris* and was occasionally found on *P. cataphracta, P. arctivorens* and *P. frigida*. The parasite larvae usually emerged from the host about the time pupation seemed imminent. Immediately after emergence they spun up in white cocoons which were arranged parallel to one another and bound together in a compact mass. It was not uncommon for the host to remain alive for 24 hours after the parasites had emerged. Cocoon masses, from which the adults emerged during late July and August, were found in the soil and in the feeding burrows of their host. The time and method of egg deposition was unobserved, but it is assumed that the borers are attacked when quite small. In two instances, *A. papaipemae* was reared from borers that had been under observation for approximately thirty days. Bird (Meusebeck, 1920) has bred this species from *P. nebris* and *P. maritima* at Rye, New York.

APANTELES LAEVICEPS Ash.

This species was moderately abundant on *Luperina stipata*, but was not reared from any of the other borers, which was probably due to the fact that *Luperina* larvae have a number of habits in common with many of the cutworms, upon which *A. laeviceps* is a common parasite. The parasite larvae emerged from full-grown borers late in June and immediately spun up in dirty white cocoons. These cocoons were bound together in very irregular masses, of between 20 and 40 each. The time spent in the cocoon varied between 6 and 15 days and averaged 9.3 days. Adults emerged July 1 to 10.

APANTELES MILITARIS (Walsh)

Two larvae of *Luperina stipata*, two of *Oligia fractilinea* and one of *Macronoctua onusta* were attacked by this parasite. The dirty white cocoons were found in contact with the bodies of the dead hosts on June 26 *(L. stipata)* and August 25 *(M. onusta)*. The adults emerged July 1-3 and August 27-30. *A. militaris* is a common parasite of armyworms and cutworms and was previously reported from *M. onusta* by Dietz (1928).

APANTELES HARTI Vier.

Seven specimens were reared from pupae collected in burrows containing the dead remains of half-grown *Epiblema otiosana larvae*. Adults emerged July 15-21. *A. harti* was also bred from larvae of *Pyrausta penitalis* Grote.

MICROBRACON LUTUS (Prov.)

Only three specimens of *M. lutus*, which is a common parasite of *Papaipema* spp. in some regions, were obtained in these experiments. All were reared singly from half-grown *P. nebris* larvae. Bird (1925b) mentions *M. lutus* as active on early stages of many *Papaipema* in New York, and Balduf (1929) reared this species from *A. zeae* in Illinois. The latter gives many interesting observations on its life history and habits.

MICROBRACON FURTIVUS (Fyles)

Two *Papaipema nebris* larvae with ectoparasites feeding upon them were collected on August 29, 1926. On September 6 the parasite larvae had completed development and spun cocoons. Adult *M. furtivus* emerged from these cocoons May 14-16, 1927.

MICROBRACON CAULICOLA Gahan

Microbracon caulicola, one of the most common parasites of the smartweed borer *(Pyrausta ainsliei* Hein.) seldom attacks borers in plants other than smartweed. In two instances, however, half-grown larvae of *Papaipema nebris*, taken from plants of *Ambrosia trifida* surrounded by borer-infested smartweeds, were parasitized by *M. caulicola*. Four larvae of *Epiblema otiosana* taken in *Bidens* sp., also associated with smartweed, were attacked by this species.

EPIURUS PTEROPHORI (Ashm.)

One specimen was bred from a small larva of *Papaipema nebris*, and two adults were reared from pupae found with the remains of half-grown *Achatodes zeae* larvae in elder (*Sambucus* sp.) stems. This species was, however, a fairly common parasite of *Epiblema otiosana*, *E. strenuana* and *Pyrausta ainsliei*. Adults were reared from *Epiblema* larvae and pupae of the summer broods and from *Epiurus* larvae found hibernating in old burrows of the host.

BASSUS SIMILLIMUS Cress.

Several specimens were reared from mature larvae of *Epiblema otiosana* and *E. strenuana* during late July and August. According to Muesbeck (1927), this species has been reared as a parasite of *Lixus scrobicollis*, a Curculionid attacking ragweed. He suggests that it probably attacks Lepidoptera and Coleoptera in stems of herbaceous plants. It seems strange, therefore, that it was not taken on *Papaipema* in ragweed.

MACROCENTRUS PALLISTERI Degrant

This species was a very common parasite on both generations of *Epiblema otiosana*, and a few specimens were reared from *E. strenuana*. *Eupteromalus viridescens* was frequently reared as a secondary parasite from the cocoons of *M. pallisteri*. Muesbeck (1932) gives *Epiblema otiosana*, *E. scudderiana* and *E. strenuana* as recorded hosts of this species.

MACROCENTRUS DELICATUS Cress.

Five adults of *M. delicatus* were bred from mature first generation larvae of *Epiblema strenuona*. Muesebeck (1932) gives a rather extensive host list for this species, which includes *Papaipema nebris* and a number of important economic pests. Allen and Lott (1930) have pointed out the importance of *E. strenuana* as a reservoir for several parasites of *Laspeyresia molesta*.

EUPTEROMALUS VIRIDESCENS (Walsh)

On September 9, 1930, eleven specimens of *E. viridescens* appeared in a cage which contained field collected pupae of *Papaipema nebris*. A hasty search for the pupa from which they emerged was fruitless, but in as much as there were no parasite pupae in the cage it was presumed that they came direct from the *Papaipema* pupae. In no other case did *E. viridescens* suggest itself as a primary parasite of the borers. Bird (1927), however, has observed such an occurrence. In his discussion of this species he says, "However, their adaptability is evident since occasionally, when no *Microplitis* cocoons are to be found in the *Papaima* burrow and when the central figure has pupated therein, the little *viridescens* are not deterred from ovipositing in the larger host, thus assuming a primary rôle."

Eupteromalus viridescens undoubtedly has several generations a year. Adults begin emerging from overwintering cocoons of *Microplitis gortynae* and *Microbracon caulicola* about the first of May, and this

emergence sometimes continues well into June. During the summer months they are practically always present and may be reared at various times from cocoons of *Microplitis gortynae, Macrocentrus pallisteri, Apanteles papaipemae* and *Microbracon caulicola.* Balduf (1929) reared it from *Microplitis gortynae* and *Microbracon lutus.*

ADDITIONAL RECORDS FROM LITERATURE

Parasites of the borers considered in this paper, which have been reported by other workers but not encountered in the course of these investigations, are listed under the names of their respective hosts.

PAPAIPEMA NEBRIS (Gn.)

Exorista sp., Washburn (1910)
Chatopsisaenea Wied., Washburn (1910)

PAPAIPEMA spp.

Hemiteles sp., Bird (sevedal papers)
Amblyteles sclestus (Cress.), Bird (1926)

ACHOTODES ZEAE (Harris)

Aphiochaeta aletiae (Comstock), Balduf (1929) (Scavenger)
Amblyteles sclestus (Cress.), Balduf (1929)
Amblyteles consignatus (Cress.), Balduf (1929)
Amblyteles caeruleus (Cress.), Balduf (1929), Breakey (1930)
Amblyteles brevicinctor (Say), Breakey (1930)
Miotropis clisiocampae Ashm., Balduf (1930)
Psychaephagus omnivorus (Walker), Breakey (1930)
Ephialtes aequalis (Prov.), Breakey (1930)
Habrocytus sp., Balduf (1929) (May be secondary)
Eurytoma sp., Balduf (1929) (Host uncertain)

MACRONOCTUA ONUSTA Grote

Miospila meditabunda Fab., Breakey (1929)
Sarcophaga latisterna Park, Breakey (1929)
Amblyteles rubicundus (Cress.), Breakey (1931)
Psychophagus omnivorus (Walk.), Breakey (1931)

EPIBLEMA STRENUANA (Walker)

Macrocentrus ancylivora Roh., Allen and Lott (1930)
Glypta rufiscutellaris Cress., Allen and Lott (1930)
Pristomerus ocellatus Cush., Allen and Lott (1930)
Cremastus minor Cush., Allen and Lott (1930)

DISCUSSION AND SUMMARY

The stalk borers are attacked by a large number of parasites of very diverse habits. They seem to attract and focus upon themselves the attacks of the natural enemies of many unrelated species which have similar

feeding habits as well as the attacks of parasites of closely related species which have very dissimilar habits. For instance, the two borers of the family Olethreutidae are attacked not only by species such as *E. pterophori*, *Bassus simillimus* and *Microbracon caulicola*, which use other stalk borers as host, but also by species such as *Lixophaga variabilis* and *Macrocentrus delicatus*, which are parasites of *Laspeyresia molesta*, *Carpocapsa pomonella* and other fruit infesting insects. Likewise the borers of the family noctuidae are the victims of *Masicera senilis*, *Microplitis gortynae* and *Epiurus pterophori*, which prey largely upon stalk boring insects, and are also attacked by such species as *Winthemia rufopicta*, *Meteorus vulgaris*, *Apanteles militaris* and others which are well known parasites of external feeding noctuids such as cutworms and armyworms.

The *Papaipema* and other noctuid borers when exposed, as they are during periods of migration, are readily attacked by cutworm parasites which would not and possibly could not attack them in their burrows. It is interesting to observe that *Luperina stipata*, a borer which works upward from the base of the plant and spends much of its life below the surface of the soil, is highly parasitized by *Meteorus vulgaris* and *Apanteles laeviceps*, two very common parasites of many cutworms. *Oligia fractilinea*, which often feeds in the open heart of the corn plant, yielded four species of parasites, all of which are reported as parasites of the amryworm.

The presence of a certain amount of filth in and about the burrows of the borers seems to attract several omnivorous flies (*Sarcophaga* spp. and *Muscina* spp.), which at times assume a parasitic habit.

A number of important factors influence the intensity of parasitism. In the first place, few of the parasites are specific to the host. Most of the borers under discussion have a single generation each year, whereas many of the parasites have two or more generations. In many cases this requires the presence of an alternate host and tends to decentralize the parasite population by scattering it over larger areas and often into different ecological habitats. On the other hand, any condition, such as a concentration of borers in a small plant or the mowing of infested plants, which produces a borer migration, increases the probability of attack by the cutworm parasites; also a large population of these parasites built up by cutworm or armyworm outbreaks will be reflected in an increased attack upon the borers.

Secondary parasites, particularly *Eupteromalus viridescens* in hymenopterus pupae and *E. dubius* in dipterous puparia, greatly retard the incidence of parasitism by the primary parasites.

The burning of fence rows and egg infested grasslands during the winter months, as recommended for the control of the noctuid borers which overwinter in the egg stage, is not particularly disastrous to the parasites, a good many of which are then safe below the surface of the soil. Others pass the winter in alternate hosts and even those hibernating in the plants are not all destroyed. A rapid fire consumes the grass, weed leaves, and small dried plants, but the large stalks of ragweed frequently are charred only on the outside. Live pupae of *Gymnochaeta ruficornis*, *Lissonota brunnea* and *Microplitis gortynae* have been taken from stumps of ragweed after a fire had burned over the area.

In addition to the parasites a large number of birds, certain mammals, predatory insects and disease destroy many borers. The predators of

Luperina stipata and Papaipema nebris have been briefly discussed in previous papers (Decker, 1930, 1931).

Synoptic table of hosts and parasites

PARASITE	Host											
	Papaipema nebris	Papaipema cataphracta	Papaipema arctivorens	Papaipema purpurifascia	Papaipema frigida	Luperina stipata	Achatodes zeae	Macronoctua onusta	Oligia fractilinea	Archanara subcarnea	Epiblema otiosana	Epiblema strenuana
Diptera												
Masicera senilis	XO	XO	X	X	XO	X	XO	XO			XO	X
Erycia deckeri	X											
Gymnochaeta ruficornis	X	X	X									
Winthemia rufopicta	X	X				X			X			
Lixophaga variabilis	X	O									X	X
Muscina stabulans	X			X			X	XO		O	X	
Muscina assimilis	X			X				O				
Sarcophaga helicis	X						X	O				
Sarcophaga cimbicis	X		X					O	X		X	
Hymenoptera												
Ectopimorpha luperinae						X						
Microplitis gortynae	XO	XO		X	XO	X	XO					
Meteorus vulgaris						X						
Sagaritis oxylus	XO								X			
Lissonota brunnea	XO	X				X	XO					
Amblyteles jucundus	X					X		XO				
Amblyteles laetus	XO	O						O	X			
Apanteles papaipemae	XO	X	X		X							
Apanteles laeviceps						X						
Apanteles militaris						X		XO	X			
Apanteles harti											X	
Microbracon lutus	XO						O					
Microbracon furtivus	X											
Microbracon caulicola	X										X	
Epiuris pterophori	X						XO				X	X
Bassus simillimus											X	X
Macrocentrus pallisteri											XO	XO
Macrocentrus delicatus	O											XO
Eupteromalus viridescens	XO											

X = Observed at Ames.
O = Reported in literature.

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EIMERIA BECKERI N. SP., A NEW COCCIDIUM FROM THE GROUND SQUIRREL, CITELLUS PYGMAEUS

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The first coccidium described from ground squirrels was *Eimeria citelli* Kartchner and Becker, 1930, which has for its host an American ground squirrel, *Citellus tridecemlineatus*. The principal characters of this parasite are tabulated in the second column of table 1. A peculiarity of the sporulating oöcyst was the large residual body that appeared among the sporoblasts but diminished rapidly in size as sporulation proceeded. The same species was observed in *Citellus pygmaeus* by Sassuchin and Rauschenbach (1932) in the Western Kazakstan (U.S.S.R.). The characters noted by these workers are tabulated in the third column of table 1. They also described from the same host another coccidium, *Eimeria volgensis*, the characters of which are presented in the sixth column of table 1.

Still another Eimeria from ground squirrels of the genus Citellus is *Eimeria beecheyi* Henry, 1932, from a California host, *C. beecheyi*. Its characters appear in the fifth column of table 1. This species has no residual body in the sporulated oöcyst, but instead there may be noted a rather large polar granule. Henry (1932) also described two species of coccidia from *Callospermophilus chrysoderius*. The names and characters of these are shown in the seventh and eighth columns of table 1.

Iwanoff-Gobzem (1934) failed to detect any coccidia in eleven ground squirrels of the species *Citellus rufiscens* from Northern Kazakstan.

Our own investigation concerns two types of oöcysts found in the feces of *Citellus pygmaeus* Pall. from Crimea, examined according to the Darling method.

The first type of oöcysts was considerably less in numbers than the second. Most of them were round, though oval forms were frequent and oviform ones rare. They were colorless, but the membrane could be seen very distinctly. Measurements were as follows: round forms, 14.4μ-23.4μ in diameter, average 16.9μ; oval forms, 17.1μ-22.5μ x 14.4μ-21.7μ, average 19.2μ x 16.8μ. The form index was 1:0.81-0.96, average, 1:0.87. The oöcyst membrane was 0.9μ in diameter, and was without a micropyle. During the formation of the spores there is a large residual body measuring 7.3μ-9.9μ. Each sporocyst likewise shows a residual body. The sporoblasts measure 5.4μ in diameter, and the spores 7.2μ x 5.4μ. There is no polar granule in the oöcyst. Oöcysts of this type correspond closely with the descriptions of *Eimeria citelli* Kartchner and Becker, 1930, as given by the describers and by Sassuchin and Rauschenbach (1932).

The oöcysts of the second type were numerous, and differed markedly from those of the first type. The color was yellow or yellowish. The membrane is thicker and covered with minute fragments from the feces. The oval shape predominates, but round forms are common. Of sixteen round specimens, eight measured 18μ across, six 19μ, one 20.7μ, and one

TABLE 1. *A comparison of the oöcysts of the coccidia described from ground squirrels*

Name of coccidia	Eimeria citelli			Eimeria beecheyi
Animals	Citellus tridecemlineatus	Citellus pygmaeus	Citellus pygmaeus	Citellus beecheyi
Authors	Kartchner & Becker, 1930	Sassuchin & Rauschenbach, 1932	Ourselves, 1934	D. Henry
Oöcyst: Shape	elliptical, oval, subspherical	elliptical, oviform, round	oval, subspherical, round	oval
Color			colorless	colorless
Size in μ	15–23 x 14–19; aver. 18.8 x 15.8	17.4–26.1 x 14.9–19.6; aver. 21.1 x 17.2	ovals: 17–21.6 x 14.4–18; aver. 19.2 x 16.8. Rounds: 14.4–23.4; aver. 16.9.	16–22 x 12.8–10.2 (sic) aver. 19.2 x 16.0.
Micropyle	0	0	0	0
Envelope	triple	triple 0.5–0.8	+ 0.9	double 1.0
Residual body	+ large	+	+ 6.3–9.9	0
Polar granule	0	0	0	0
Form index			0.81–0.96; aver. 0.87	
Spores: Size	5.2–9 x 3.9–7	6–9.4 x 3–4.3	7.2 x 5	
Sporozoites: Size	5–7.5 x 1.75–3.3	5.2–8.7 x 1.8–3.4		
Sporulation time	72 hours	72 hours		4–5 days

TABLE 1. *(Continued)*

Name of coccidia	*Eimeria volgensis*	*Eimeria callosphermophili*	*Eimeria bilamellata*	*Eimeria beckeri* n. sp.
Animals	*Citellus pygmaeus*	*Callospermophilus chrysoderius*		*Citellus pygmaeus*
Authors	Sassuchin & Rauschen-bach, 1932	D. Henry		Ourselves, 1934
Oöcyst: Shape	oviform	sub-spherical	oviform	oval, round
Color		yellowish		yellowish, yellow
Size in μ	23.2–31.9 x 17.4–27.6; aver. 27.2 x 21.9	16–23 x 16–22.4; aver. 19.2 x 15	26.5–35.6 x 22.4–25.6, aver. 32 x 25.6	ovals; 19.8–22.5 x 16.2–19.5; aver. 21.2 x 18.2. Rounds: 18–21.6; aver. 19
Micropyle	+	0	+	0
Envelope		double	double	double
Residual body		+	0	0
Polar granule		0	0	0
Form index				0.70–0.91; aver. 0.85
Spores: Size		10.2 x 8.5	16 x 9.6	
Sporozoites: Size				
Sporulation time				

21.6μ; average 19.0μ. The oval forms measured from 19.8μ-22.5μ x 16.2μ-19.5μ; average size, 21.2μ x 18.2μ; most frequent size (27 out of 50 specimens), 21.6μ x 18.0μ. The form-index was 1:0.70-0.90; average, 1:0.85; most frequent, 1:0.83.

There is no residual body in the oöcyst during sporulation, but one appears in each sporocyst. Neither is there a polar granule in the oöcyst.

The oöcysts of the second type distinguish themselves from *Eimeria citelli* by the absence of an oöcystic residual body, by their yellow color, and by the absence of a double membrane. They differ from *E. beecheyi* in color, in the absence of a polar granule, and still further by the fact that they produce some round forms, which seem not to occur in *E. beechyi*.

We name this coccidium *Eimeria beckeri* in honor of the American parasitologist, E. R. Becker, who has worked much with the Coccidia.

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PLATE I

Sporulated Oöcysts of Coccidia from *Citellus pygmaeus*.
Fig. 1. *Eimeria citelli.*
Fig. 2. *Eimeria beckeri* n. sp.

PLATE I

Fig. 1.

Fig. 2.

THE MITOGENETIC EFFECT ON YEAST OF OLIGODYNAMIC
RADIATION FROM METALS

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The "oligodynamic effect" of the heavy metals on micro-organisms is familiar through its practical application in water purification. The term was suggested by Nägeli for the toxicity of metals at extreme dilution and has since been extended to include similar effects produced by "metals at a distance." While the oligodynamic effect is easily produced and readily recognized, oligodynamic action is not satisfactorily explained by the theories that have been advanced. These theories, according to Buchanan and Fulmer (1930), are of two types, ". . . the one associating it with various theories of emanations, imponderable agents and the like and the other based upon the working of colloidal phenomena, especially adsorption." Much of the evidence upon which these theories rest is confusing because of complex and ambiguous experimental conditions. An attempt has been made in this laboratory to simplify conditions and limit possibilities in oligodynamic experiments through the use of a liquid medium of known composition, small seedings of pure culture yeast, and metals placed outside silica or·glass containers at a distance of 1 to 2 mm.

EXPERIMENTAL

The medium used in this investigation was prepared by dissolving 5 g. sucrose; 2 g. monopotassium acid phosphate; 2.3 g. magnesium sulphate; and 0.2 g. calcium chloride in one l. distilled water. The sucrose and salts were "chemically pure" and the water was redistilled from glass apparatus. The solution was sterilized by heating it to boiling in plugged Pyrex flasks on three successive days and was then stored. The yeasts used were well acclimated to this medium and grew slowly but satisfactorily in it. Seeding suspensions, made from two-day-old cultures in the medium, were of a cell concentration such that one or two drops could be used for the inoculation of a measured volume of medium. The initial cell concentration of the cultures, + or − 1 cell per unit volume, varied in different sets but was approximately the same in all the tubes of any one set. Changes in cell concentration were estimated by count in a Levy hemocytometer, one of the nine large squares serving as the unit of volume. The cultures were incubated at 28°-30°C. and counts were made at stated intervals.

EFFECT OF METALS ON YEAST CULTURES

Preliminary experiments on the oligodynamic effect of metals on yeast cultures enclosed in glass were made in 50 cc. Erlenmeyer flasks

with glass caps over the cotton stoppers. As soon as inoculated, these flasks were placed on pieces of freshly cleaned sheet copper, iron, lead or aluminum and put in an incubator together with control flasks on a cardboard tray. The daily cell-count for the flasks incubated on the metal trays was higher than that for the control. When counting became difficult because of increased cell concentration, the cultures were poured into test tubes and their turbidity compared by Peskett's method (1927). The turbidity of the tests was without exception greater than that of the controls. Similar results were obtained with two strains of *Saccharomyces cerevisiae,* two strains of *S. ellipsoideus,* a cider yeast "K," a top distillery yeast "XII" and a yeast isolated from a commercial yeast cake. In these experiments there was no doubt as to the difference between multiplication in the control and test cultures. It also seemed reasonably certain that the cause of the difference emanated from the metal trays and similarly affected different yeasts.

In the following experiments, tubes and individual metal jackets were substituted for flasks and metal trays. The metal jackets were one and one-half inch lengths of brass, steel or lead tubing, aluminum extraction thimbles, and cylinders of heavy silver foil of approximately five-eighths inch in diameter, in which the culture tubes fitted loosely. Holders for these were wood blocks each with six holes with a metal disk at the bottom. When in the jacket, the distance of the culture from the metal was the thickness of the tube wall plus the air space between the tube and its jacket. The distance was not uniform but was approximately 1 to 2 mm. Five cc. of sterile medium was measured into sterile tubes and as nearly as possible, equally inoculated from a suspension of yeast "K". The tubes to serve as test cultures were placed in holders with metal casings, the controls in one without, and the loaded holders put in the incubator. Cell counts were made after twenty-four, forty-eight, and seventy-two hours.

COMPARISON OF QUARTZ AND GLASS CULTURE TUBES

The object of the following experiment was to determine the relative merits of quartz and glass for the transmission of the activating agent from the metals. The culture vessels tested were Vitreosil test tubes 0.5 x 4.0 in.; glass vials, 0.75 x 3.24 in.; ordinary glass test tubes, $\frac{5}{8}$ x 5 in.; and thin-walled glass culture tubes $\frac{5}{8}$ x 6 in. Cell counts were made at twenty-four- hour intervals. Those recorded in table 1 are averages for the six cultures in each holder. The percentage gain or loss was calculated from these averages.

Cell multiplication in the three kinds of culture vessels exhibited different degrees of oligodynamic influence. The cell increase in twenty-four hours in the jacketed Vitreosil tubes was less than in the controls; in the glass vials there was a fairly uniform gain over the controls; and in the thin-walled glass culture tubes the gains were significant. These results are interpreted as indicating that quartz transmitted from the metals enough of a stimulating factor to be repressive to multiplication but not lethal to the cells; while that which glass transmitted was probably different in both quality and quantity and produced a milder stimulation and beneficial effects.

TABLE 1. *Comparison of vitreosil tubes, glass vials and glass tubes*

	Control				Brass				Steel				Lead				hrs.
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	
Vitreosil Tubes	6	88	299		4	98	504		4	85	464		4.5	95	332		Ave. no. cells per unit
					− 33	+ 11	+ 68		−33	− 3.4	+ 55		−25	+ 8	+ 11		P'c't'g. Inc.
Glass Vials	4.3	73	484		5.8	94	808		6	96	648		6.6	95	696		cells
					+ 35	+ 28	+ 67		+39	+31	+ 33		+53	+30	+ 45		Percentage
Glass Tubes	4.6	109	516		6.2	110	612		5	67	520		7.1	80	768		cells
					+ 33	− 0.8	+ 18		+ 4.7	−38	+ 0.8		+57	−26	+ 48		Percentage
Glass Culture Tubes	4	73	230	262	8.5	78	317	402	7.5	76	278	390	7.7	76	289	389	cells
					+112	+ 6	+ 38	+ 53	+87	+ 4	+ 21	+ 45	+92	+ 4	+ 26	+ 48	Percentage

CULTURES INCUBATED IN METAL JACKETED GLASS TUBES

Since in the experiment above the effect of the metals on the cultures in glass tubes was pronounced and on the whole positive rather than negative and since the response of the different yeasts tested was apparently the same, glass culture tubes and yeast K were chosen for use in this and the following experiment. The procedure was the same as that given in detail in the test of quartz and glass tubes. The cultures in thin-walled glass tubes were incubated in copper, steel, lead, aluminum and silver jackets at 28° to 30°C. The length of exposure of the culture to the action of the metal corresponded to the time of cell counts which were made at twenty-four hour intervals. The percentage gain or loss was calculated from average counts as in table 1. The results expressed in percentage are given in table 2.

The figures in table 2 show for twenty-four hours an irregular beneficial effect of the metal jackets upon cell multiplication, and for forty-eight hours an inhibitive effect. Under the conditions of the experiment, the influence of the metals may be assumed to have continued throughout the period of incubation. However, additional factors must certainly affect cell multiplication in the later stages of culture growth. It is only at low cell concentration, when mitogenetic radiation (Borodin, 1930. Gurwitsch, 1932) and other agencies are least and, unfortunately, the percentage of error greatest, that the influence of the metals can be estimated. The results of this experiment established the fact of the sensitiveness of yeast cells to a probable oligodynamic radiation from metals separated from the culture by glass walls. Taken as a whole, the experiment indicates the metal as the source of a variable radiation, but furnishes no definite information as to its cause or classification.

EFFECTS OF METALS ON THE MEDIUM

In the experiments already described the cells and medium were alike exposed to the radiation from metals. From the results of these no opinion could be formed as to whether the radiation reached the cells directly, or was transmitted or absorbed by the medium. In order to test these possibilities, sterile glass culture tubes containing 5 cc. of medium were placed in the metal jackets and allowed to stand in closed cupboards at laboratory temperature. At the expiration of a given time, the tubes of medium were transferred to racks and, together with the controls, inoculated, incubated and counted as already described. The results expressed as percentage gain or loss are recorded in table 3.

In spite of the fact that the results in table 3 are characterized by irregularities, they do furnish evidence of the reception by the medium of radiation from the metal jackets. In this experiment the metal casings were not protected from the atmosphere of a chemical laboratory. Under these conditions the variety and extent of the reactions which must have taken place on the surface of the metals become matters of conjecture. If it is assumed that the radiations were caused by these devious reactions, irregularity in results is to be expected.

A superficial test of the correctness of this assumption was made by substituting closely fitting jackets of metal foil for the loosely fitting casings of metal tubing. A clean strip of copper foil, about one and one-half

TABLE 2. Cultures in metal jacketed glass tubes

Brass				Steel				Lead				Aluminum				Silver				hrs.
24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	
+73	+22	+26	+63	+58	+23	+24	+70	+78	+22	+12	+32	+78	+48	+43	+60					Pctg. Inc.
+133	0	+33	+54	+63	0	+1	+30	+76	0	+24	+71	+77	+.5	+17	+96					Pctg.
+126	+2	+54		+52	−8	+7		+20	+7	+12		+72	+1	+41	+32					"
+31	−19	0	+24					+8	−19	−11	+29	+10	−28	+17						"
+28	+25	+20		−25	−28	−7		−35	−15	−26		−48	−30	+6		+26	+26	+7		"
+101	+5	+46	+40	+51	+10	+18	+40	+61	+2	−5	+28	+80	+25	+35	+89	+47	+26	+32	+39	"
+140	+19	+52	+92	+48	+2	+26	+59	+41	−9	+6	+35	+70	+15	+40	+91	+39	+19	+21	+45	Pctg.

TABLE 3. *Medium exposed in metal jacketed glass tubes*

Days exposed	Brass				Steel				Lead				Aluminum				Silver				hr.
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	
3	0	+ 42	− 4	−14	0	+44	− 8	−14	−27	− 29	−27	−33	− 7	− 8	+ 12	−22	+ 6	+ 20	+ 27	+11	Pctg.
4	+26	− 35	−26		+ 14	−55	+ 1	+2	+51	+ 16	+12		−56	− 20	− 41		− 1.5	+ 31	− 18		,,
4	+80	+ 32	+26		+141	+20	+62		+67	+130	+53		+16	+ 16	+ 26		+39	+ 16	+ 16		,,
5	0	+135	+35		− 1	+88	+40						+ 8	+ 8	+ 17			+ 19	+ 35		,,
7	+46	+114	+38		− 17	−31	−50		−16		+52		−29	+147	+140		+67	+148	+211		,,
7	+25	+ 61	+66										+43	+ 41	+ 32						,,
7	+14	+ 36	+30										−39	− 6	+ 30						,,

inches wide, was tightly wrapped around a glass test tube and the lower edge bent in to cover the bottom as smoothly as possible. The foil jacket was held in place by a covering of gummed paper pasted to the tube at the upper edge. Other tubes were similarly jacketed with silver foil and others were imbedded in melted lead in shorter tubes of larger diameter. The chances of chemical action between the metals and the atmosphere were thus appreciably reduced. Two tubes in platinum cylinders were added. Both cultures and medium were exposed in these tubes and in tubes in metal casings to serve as controls. Unexposed controls were included when the cultures were incubated. Cell multiplication in the foil jacketed tubes differed much less from that in the unexposed controls than from that in the exposed controls. The gain or loss for the first twenty-four hours in the platinum cylinders varied for the exposed cultures from + 12 per cent to + 5.6 per cent and for the exposed medium from + 5.1 per cent to − 2 per cent. Under the conditions of the experiment the results obtained cannot be regarded as proof of the correctness of the assumption that incidental chemical reactions on the surface of the metals were responsible for the radiation, but may be considered as evidence in favor of it.

EXPOSURE OF THE MEDIUM IN GLASS FLASKS

In order to secure a more uniform exposure of the medium, glass flasks instead of tubes of medium were placed in metal holders of various kinds and allowed to stand in closed cupboards at laboratory temperature for longer or shorter periods. The metal holders used in this experiment were an iron mortar, a copper retort in two sections, and a deep lead dish hammered from heavy sheet lead. The flasks chosen were of thin glass and of shape and size to fit snugly into the metal holders. They were sterilized, filled with sterile medium to the top of contact with the metal, plugged with cotton and capped or stoppered with glass, and occasionally shaken during exposures of from ten to thirty days. The exposed medium was measured into culture tubes, ten for each set, and inoculated together with controls of unexposed medium. The exposed medium was tested unheated, after heating to boiling, and after standing in the refrigerator and at room temperature. Inoculation was the same for a single series represented in table 4 by percentages on the same horizontal line.

Cell counts in the tubes of the same set were more nearly alike for cultures in medium which had been exposed in bulk than in medium which had been exposed in individual tubes. This would indicate a greater uniformity in the irradiation of the medium when exposed in bulk. If the radiations were constant, then the percentage gain or loss in cell increase should be comparatively uniform for a given length of exposure. But the figures for ten-day exposures in the forty-eight hour column of table 4 range from + 110 per cent to + 228 per cent for copper and from + 22 per cent to + 131 per cent for iron. This may be accepted as evidence in favor of the view that uncontrolled chemical reactions on the surface of the metals might be responsible for the radiation. (Braunstein, 1932. Potozky, 1932.)

It is further shown by the results in table 4 that heating the exposed medium to boiling did not destroy the effect of the radiation. Some deterioration on standing is indicated by the results of No. 7 and No. 8. In

TABLE 4. *Medium exposed in glass flasks*

No.	Days exposed	Copper Unheated 24	48	72	Copper Heated 24	48	72	Iron Unheated 24	48	72	Iron Heated 24	48	72	Lead Unheated 24	48	72	Lead Heated 24	48	72	hrs.
1	10	+52	+110					+30	+22					−48	+8		−46	−5		Pctg. Inc.
2	10	+87	+228					+80	+131					−45	−14	+51	−2	+14	+28	Pctg.
3	10																			"
4	10							+52	+86	+44	+49	+81	+41							"
5	10	+62	+141	+55	+56	+130	+49	+37	+26	+20										"
6	10	+82	+190	+61	+77	+181	+56													"
7		+71	+176	+54	+67	+160	+60													"
8					+64	+142	+48													"
9	15	+29	+20	+34	+76	+22	+30							−1.3	+2		−1.7	+16		"
10	15																			"
11	20	−18	−31	−62	−7	−8	−12													"
12	10	+74	+120	+63	+69	+100	+58													"
	15				+20	+17	+22													"
	20				+3.8	+12														"

these tests the unused medium from No. 6 was stored for three weeks, that for No. 7, unheated, in a refrigerator and that for No. 8, heated, at room temperature. In Nos. 12, 13 and 14, the medium was exposed in a copper retort for ten days and a portion then removed for use in No. 12. The remainder of the medium was heated to boiling, returned to the retort for five days and a portion removed for use in No. 13. The remainder was again heated and after an additional exposure of five days was used in No. 14. The results obtained in this test and those of No. 11 for copper, and those of Nos. 2 and 3 for lead suggest the possibility of over-irradiation of the medium.

SUMMARY

It has been demonstrated that the multiplication of yeast cells in a synthetic medium may be influenced by metals placed in close proximity but outside quartz or glass containers. It has also been demonstrated that the mitogenic effect is qualitatively the same, whether the cells and medium, i. e. the culture, are irradiated or the medium alone is exposed and subsequently inoculated. It would seem reasonably certain that the mitogenetic rays came directly or indirectly from the metals. It follows that oligodynamic action, as exhibited in these experiments, is a radiation phenomenon.

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THE DEFLECTION OF AN ISOTROPIC RECTANGULAR PLATE UNDER THE ACTION OF CONTINUOUS AND CONCENTRATED LOADS WHEN SUPPORTED AT TWO OPPOSITE EDGES[1]

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Herein is given the solution for the bending of an isotropic rectangular plate of constant thickness, simply supported at two opposite edges and subjected to different types of normal loads. Mathematically, the thin plate is treated as a plane surface in the unstrained state and the energy stored in the plate in the strained state is considered as due to flexural stresses.

In the general case one pair of edges is supported or pinned so that no deflections or moments are permitted, and the other pair of opposite edges is entirely free. In this case a uniformly loaded rectangular area is applied normally to the plate. The results are given for a finite rectangle as well as for a rectangle of infinite length (designated infinite plate strip.) By a suitable limiting process the loaded area may be shrunk in such a manner as to become a continuous line load or even a concentrated load. In the closing section the case of a plate with one pair of edges pinned and one pair clamped is obtained from the earlier case by a special device.

EQUILIBRIUM EQUATIONS

The initially plane middle surface of the plate will be taken as the plane of the rectangular cartesian coordinates x,y and the edges of the plate will be segments of the lines $x = o$, $x = a$, $2y = \pm b$, so that we have a plate of dimensions a by b symmetrical with respect to the x axis. The plate is considered to be of small uniform thickness 2h, and the flexural rigidity N of the plate is given by

$$N = \frac{2Eh^3}{3(1-\mu^2)}, \quad \dots\dots\dots\dots\dots\dots\dots (1)$$

where E is Young's modulus and μ is Poisson's ratio.

The plate is subjected to a uniform pressure p, applied normally to one of its faces over a rectangular area 2 c by 2 d, whose sides are parallel to the edges of the plate and whose centroid is at x_o, y_o (fig. 1). The deflection $w = w(x,y)$, or the displacement of a point on the middle surface in the direction of the pressure p, is to be determined so that the equilibrium equation
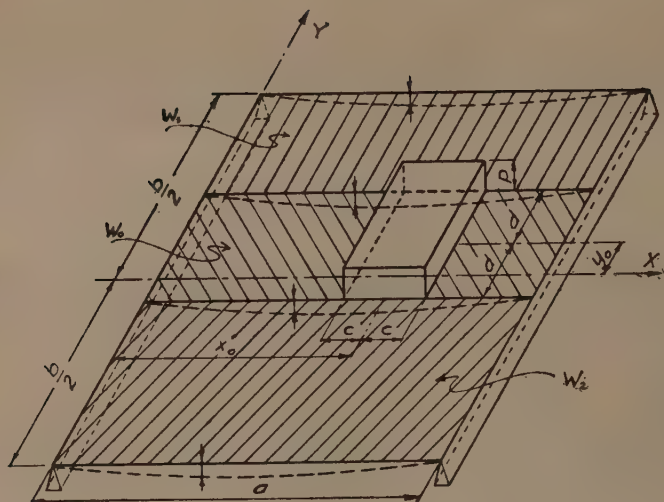
Fig. 1

$$\nabla^4 w = \frac{\partial^4 w}{\partial x^4} + 2\,\frac{\partial^4 w}{\partial x^2 \partial y^2} + \frac{\partial^4 w}{\partial x^4} = \frac{P}{N} \quad \text{... (2)}$$

is satisfied at all points in the region $0 \leqq x \leqq a,\ -b \leqq 2y \leqq b$, as well as certain further boundary conditions. As indicated in the figure, the right member of (2) is zero everywhere except in the region $x_0 - c \leqq x \leqq x_0 + c,\ y_0 - d \leqq y \leqq y_0 + d$, where it is a constant.

The conditions to be satisfied at the supported edges $x = 0,\ x = a$ are

$$w(0,y) = w(a,y) = \nabla^2 w(0,y) = \nabla^2 w(a,y) = 0, \quad \text{................ (3)}$$

where ∇^2 stands for $\left(\dfrac{\partial^2}{\partial x^2} + \dfrac{\partial^2}{\partial y^2} \right)$. These conditions express the van-

ishing of the deflection and flexural moments. At the free edges $2y = \pm b$, the vanishing of the transverse forces R_y and of the flexural couples M_y in planes normal to these free edges is expressed by

$$\left. \begin{aligned} M_y &= -N\left[\frac{\partial^2 w}{\partial y^2} + \mu\,\frac{\partial^2 w}{\partial x^2} \right] = 0 \\[2mm] R_y &= -N\left[\frac{\partial^3 w}{\partial y^3} + (2-\mu)\,\frac{\partial^3 w}{\partial x^2 \partial y} \right] = 0\,. \end{aligned} \right\} \text{....................... (4)}$$

To solve the problem, the plate is divided into three regions at $y = y_0 \pm d$, of which the outer two have no normal load, while the

middle section has a uniform load of intensity p over the range $x_0 - c \leqq x \leqq x_0 + c$. Designating the deflection functions* by W_1, W_2, and W_0, for the two unloaded and one loaded portions respectively (fig. 1), one may construct a suitable function of the form[2].

$$
\left.
\begin{aligned}
W_i &= \frac{4pa^4}{N\pi^5} \Sigma \frac{1}{n^5} Y^{(i)}_n(y) \sin kx_0 \sin kc \sin kx \\[2mm]
k &= \frac{n\pi}{a}, \ (i=0,1,2), \ (n=1,2,3, \ldots \ldots),
\end{aligned}
\right\} \quad \ldots\ldots\ldots\ldots\ldots (5)
$$

where

$$
Y^{(i)}_n = \delta^{(i)}_1 + A^{(i)}_n \cosh ky + B^{(i)}_n \sinh ky + C^{(i)}_n \, ky \cosh ky +
$$
$$
D^{(i)}_n \, ky \sinh ky
$$

with $\delta^{(i)}_1 = 0$ for $i \neq 0$, and $\delta^{(i)}_1 = 1$ for $i = 0$. .... (6)

The conditions imposed upon (5) by equations (3) are satisfied identically for all values of y along the supported edges $x = 0$ and $x = a$. The three functions of (6) are so chosen that W_1 of (5) satisfies (2) in the form[3]

$$
\left.
\begin{aligned}
\nabla^4 W_i &= \frac{4p\delta^{(i)}_1}{N\pi} \Sigma \frac{1}{n} \sin kx_0 \sin kc \sin kx, \\[2mm]
(i &= 0,1,2) \ , \ (n = 1,2,3, \ldots\ldots) \ ,
\end{aligned}
\right\} \quad \ldots\ldots\ldots\ldots\ldots (7)
$$

in which the right member is zero for $i = 1,2$, and for $i = 0$ it represents a constant load function in the middle section for the range $x_0 - c \leqq x \leqq x_0 + c$ only. The functions $Y^{(i)}_n(y)$ of (6) are valid as follows:

$$Y^{(1)}_n \text{ for } y \geqq y_0 + d;$$
$$Y^{(0)}_n \text{ for } y_0 - d \leqq y \leqq y_0 + d;$$
$$Y^{(2)}_n \text{ for } y \leqq y_0 - d.$$

It remains to determine the twelve constants in (6) which are $A^{(0)}_n$, $B^{(0)}_n$, $C^{(0)}_n$, $D^{(0)}_n$ for the W_0 deflection function of the middle section bearing the load, and $A^{(1)}_n$, $B^{(1)}_n$, $C^{(1)}_n$, $D^{(1)}_n$; $A^{(2)}_n$, $B^{(2)}_n$, $C^{(2)}_n$, and $D^{(2)}_n$ for the functions W_1 and W_2 respectively in the unloaded sections of figure 1.

DETERMINATION OF CONSTANTS

Four of the necessary conditions for the evaluation of these constants are the conditions (4) at the free edges $2y = \pm b$. The remaining con-

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[2] M. Levy, Comptes Rendus, 129: 535, 1899.

[3] The use of divergent Fourier series in representing concentrated loads was introduced by A. Mesnager, Comptes Rendus 164: 600, 1917.

* In sequel the deflection function w of preceding equations will be denoted by W_0, W_1, and W_2 for the respective sections of the plate in figure 1.

ditions arise in prescribing that the surface, its slope, the flexural moments and vertical shearing forces be continuous for all values x on the lines of juncture $y = y_0 \pm d$. These conditions are equivalent to prescribing that the functions W_0, W_1, and W_2 together with their first three derivatives with respect to y be continuous. All these boundary requirements are expressed in the following equations:

$$
\begin{aligned}
&\text{At } y = y_0 + d; \quad (W_1 - W_0) = (W_1 - W_0)_y = (W_1 - W_0)_{yy} = \\
&\qquad\qquad\qquad\qquad (W_1 - W_0)_{yyy} = 0 \\
&\quad y = y_0 - d; \quad (W_2 - W_0) = (W_2 - W_0)_y = (W_2 - W_0)_{yy} = \\
&\qquad\qquad\qquad\qquad (W_2 - W_0)_{yyy} = 0 \\
&\quad 2y = b \quad\quad ; \quad (W_{1yy} + \mu W_{1xx}) = [W_{1yyy} + (2 - \mu) W_{1xxy}] = 0 \\
&\quad 2y = b \quad\quad ; \quad (W_{2yy} + \mu W_{2xx}) = [W_{2yyy} + (2 - \mu) W_{2xxy}] = 0 .
\end{aligned} \right\} \quad (8)
$$

In order to represent the values of the above twelve constants and to analyze special cases which are given in the subsequent analysis, it is found convenient to introduce the following notation:

$$
\left.
\begin{aligned}
2\Delta_1 &= (3 + \mu) \sinh kb - (1 - \mu) kb \\
2\Delta_2 &= (3 + \mu) \sinh kb + (1 - \mu) kb \\
2\Delta_3 &= (3 + \mu) \cosh kb - (1 - \mu) \\
2\Delta_4 &= (3 + \mu) \cosh kb + (1 - \mu)
\end{aligned}
\right\} \quad (9)
$$

$$
\left.
\begin{aligned}
\lambda_1 &= \sinh kd \cosh ky_0 \\
\lambda_2 &= \sinh kd \sinh ky_0 \\
\lambda_3 &= \cosh kd \cosh ky_0 \\
\lambda_4 &= \cosh kd \sinh ky_0
\end{aligned}
\right\} \quad (10)
$$

$$
\left.
\begin{aligned}
\Omega_1 &= 2\lambda_1 - ky_0\lambda_2 - kd\lambda_3 \\
\Omega_2 &= 2\lambda_2 - ky_0\lambda_1 - kd\lambda_4 \\
\Omega_3 &= 2\lambda_3 - ky_0\lambda_4 - kd\lambda_1 \\
\Omega_4 &= 2\lambda_4 - ky_0\lambda_3 - kd\lambda_2
\end{aligned}
\right\} \quad (11)
$$

With this notation the value of the constants in (6) which satisfy conditions (8) may be expressed as follows:

$$
2A^{(1)}{}_n - \Omega_2 = 2A^{(0)}{}_n + \Omega_3 = 2A^{(2)}{}_n + \Omega_2 = \frac{-\lambda_1 (\Delta_1\Delta_2 - \Delta_3\Delta_4)}{\Delta_1 (1 - \mu)} + \frac{\Delta_4\Omega_1}{\Delta_1} \quad (12)[4]
$$

$$
2B^{(1)}{}_n + \Omega_1 = 2B^{(0)}{}_n - \Omega_4 = 2B^{(2)}{}_n - \Omega_1 = \frac{-\lambda_2 (\Delta_1\Delta_2 - \Delta_3\Delta_4)}{\Delta_2 (1 - \mu)} - \frac{\Delta_3\Omega_2}{\Delta_2} \quad \begin{matrix}(13)\\(14)\end{matrix}
$$

$$
2C^{(1)}{}_n - \lambda_1 = 2C^{(0)}{}_n + \lambda_4 = 2C^{(2)}{}_n + \lambda_1 = \frac{1}{\Delta_2}\left\{ \lambda_2\Delta_4 - \Omega_2 (1 - \mu) \right\}
$$

$$
2D^{(1)}{}_n + \lambda_2 = 2D^{(0)}{}_n - \lambda_3 = 2D^{(2)}{}_n - \lambda_2 = \frac{-1}{\Delta_1}\left\{ \lambda_1\Delta_3 + \Omega_1 (1 - \mu) \right\} ,
$$

[4] In subsequent sections the equations (12), (13), and (14) refer to the three sections of the plate with deflection W_1, W_0, and W_2, respectively.

where
$$\Delta_3\Delta_4 - \Delta_1\Delta_2 = 2(1+\mu) + (1-\mu)^2 (kb/2)^2.$$

This completes the solution of the general case. The surface of the plate is completely represented by the equations (5), (6), (12), (13) and (14). The formal solution need not be written out in full for the general case, as it becomes greatly simplified in the following cases which are of special interest.

CONTINUOUS LOADS

Symmetrical Loads. Finite Rectangle. Consider the centroid of the loaded area to be upon the x axis. Then $y_0 = 0$ and equations (9) to (14) yield the following:

$$\left\{\begin{array}{l}\lambda_1 = \sinh kd; \; \lambda_3 = \cosh kd; \; \lambda_2 = \lambda_4 = \Omega_2 = \Omega_4 = 0, \\ A^{(1)}_n - A^{(2)}_n = B^{(1)}_n + B^{(2)}_n = C^{(1)}_n + C^{(2)}_n = D^{(1)}_n - D^{(2)}_n = 0.\end{array}\right\} \text{(15)}$$

Hence the functions $Y^{(1)}_n$ for $y > y_0 + d$ and $Y^{(2)}_n$ for $y < y_0 - d$ are identical and give a symmetrical deflection surface. The function $Y^{(0)}_n (y)$ for the loaded area is also an even function of y as $B^{(0)}_n = C^{(0)}_n = 0$.

When $2d = b$, the loaded portion is a strip of width $2c$ extending the entire distance between the free edges. It can be shown that the constants of (13) reduce to the following:

$$\begin{aligned}2\Delta_1 A^{(0)}_n &= \frac{\sinh (kb/2)}{(1-\mu)}\left[\left(\frac{3+\mu}{2}\right)-\left(\frac{1-\mu}{2}\right)^2 (1-k^2b^2)\right] + \Delta_4\Omega_1 - \Delta_1\Omega_3 \\ &= 2\mu\left[\left(\frac{1+\mu}{1-\mu}\right)\sinh\frac{kb}{2} - \frac{kb}{2}\cosh\frac{kb}{2}\right], \\ 2\Delta_1 D^{(0)}_n &= \sinh\frac{kb}{2}\left[\left(\frac{3+\mu}{2}\right) - \frac{3}{2}(1-\mu)\right] = 2\mu\sinh\frac{kb}{2}, \\ B^{(0)}_n &= C^{(0)}_n = 0.\end{aligned}\right\} \text{.... (16)}$$

The complete solution is given by

$$W_0 = \frac{4pa^4}{N\pi^5}\Sigma\frac{1}{n^5}[1 + A^{(0)}_n \cosh ky + D^{(0)}_n ky \sinh ky] \sin kx_0 \sin kc \sin kx.$$

$$(n = 1,2,3, \; . \; . \; . \; . \; . \;) \; .... \text{(17)}$$

For a uniform load covering the entire plate, $2c = 2x_0 = a$,

$$\left.\begin{aligned}W_0 &= \frac{4pa^4}{N\pi^5}\Sigma\frac{1}{n^5}[\, 1 + A^{(0)}_n \cosh ky + D^{(0)}_n ky \sinh ky] \sin ky. \\ &\quad (n = 1,3,5, \; . \; . \; . \; . \; . \; . \;)\end{aligned}\right\} \text{.......... (18)}$$

This solution becomes the same as that obtained by Estanave[5], who used $\mu = \frac{1}{4}$.

Infinite Plate Strip. For the case of an infinite plate strip simply supported at $x = 0$ and $x = a$, and subjected to a finite loaded area, the equations (15) are valid since $y_0 = 0$. As b becomes infinite, the following limiting values hold:

$$\lim_{b \to \infty} \frac{\Delta_1\Delta_2 - \Delta_3\Delta_4}{\Delta_1} = \lim_{b \to \infty} \frac{\Delta_1\Delta_2 - \Delta_3\Delta_4}{\Delta_2} = \lim_{b \to \infty} \frac{\Omega_1}{\Delta_1} = 0,$$

$$\lim_{b \to \infty} \frac{\Delta_3}{\Delta_1} = \lim_{b \to \infty} \frac{\Delta_4}{\Delta_1} = \lim_{b \to \infty} \frac{\Delta_3}{\Delta_2} = \lim_{b \to \infty} \frac{\Delta_4}{\Delta_2} = 1 \ . \quad \rbrace \ \cdots\cdots (19)$$

Then the solution will be given by (5) and (6) if the following values of the constants [obtained from (12), (13), (14), (15) and (19)] are used.

$$2A^{(1)}{}_n = 2A^{(2)}{}_n = -2B^{(1)}{}_n = 2B^{(2)}{}_n = 2\sinh kd - kd\cosh kd \,\rbrace$$

$$2C^{(1)}{}_n = -2C^{(2)}{}_n = -2D^{(1)}{}_n = -2D^{(2)}{}_n = \sinh kd$$

$$2A^{(0)}{}_n = (2 + kd)(\sinh kd - \cosh kd) \qquad \cdots (20)$$

$$2D^{(0)}{}_n = (\cosh kd - \sinh kd) \qquad .$$

When $d \to \infty$, $A^{(0)}{}_n = B^{(0)}{}_n = C^{(0)}{}_n = D^{(0)}{}_n = 0$, and $Y^{(0)}{}_n = 1$, the deflection surface is

$$W_0 = \frac{4pa^4}{N\pi^5} \sum_1^\infty \frac{1}{n^5} \sin kx_0 \sin kc \sin kx. \ \cdots\cdots (21)$$

If $2c = 2x_0 = a$, there is a uniform load over the entire infinite strip, and the deflection becomes that of a uniformly loaded beam[6].

$$W_0 = \frac{p}{24N}(x^4 - 2ax^3 + a^3x) = \frac{4pa^4}{N\pi^5} \sum \frac{1}{n^5} \sin kx. \quad (n = 1,3,5, \ldots \ldots) \ .$$

LINE LOADS AND CONCENTRATED LOADS

Line Loads parallel to the *Supported Edges.* For a finite rectangle the general case is applicable. If c approaches zero in such a manner that $2pc$ remains finite and equal to q, which is the linear density along the line $x = x_0$, then, when the right number of (5) is multiplied by $\frac{n\pi c}{akc} = 1$, it follows that

[5] E. Estanave, Paris Thesis 1900, "Contribution a l'étude de l'équilibre elastique d'une plaque rectangulaire mince."

[6] A. Nadai. "Elastische Platten." p. 70. Springer, 1925.

$$W_i = \frac{2qa^3}{N\pi^4} \sum_1^\infty \frac{1}{n^4} Y^{(1)}_n (y) \, \sin kx_0 \sin kx, \quad (i = 0,1,2) \quad\text{.......................} (22)$$

In (22) the values (12), (13), and (14) are used. When $2d = b$,

$$W_0 = \frac{2qa^3}{N\pi^4} \sum_1^\infty \frac{1}{n^4} [1 + A^{(0)}_n \cosh ky + D^{(0)}_n ky \sinh ky] \, \sin kx_0 \sin kx,$$

where $A^{(0)}_n$ and $D^{(0)}_n$ are given by (16).

For the infinite plate strip, the results are obtained from (20) and (22) above.

Line Loads parallel to the Free Edges. If d approaches zero in such a way that 2 pd remains finite and equal to q, the line density on $y = y_0$,

then multiplying the right member of (5) by $\dfrac{n\pi d}{akd} = 1$, we find that

$$W_i = \frac{2qa^3}{N\pi^3} \sum_1^\infty \frac{1}{n^4} Y^{(1)}_n \, \sin kx_0 \sin kx, \quad (i = 1,2) \quad\text{......................} (23)$$

in which the functions $Y^{(1)}_n$ and $Y^{(2)}_n$ have terms involving $\lambda_j/(kd)$ $(j = 1,2,3,4)$ in the constants $A^{(1)}_n, A^{(2)}_n, \ldots, D^{(1)}_n, D^{(2)}_n$. The limiting values of the required terms are:

$$\left.\begin{aligned}
\lim_{d\to 0} \frac{\lambda_1}{kd} &= \cosh ky_0; \quad \lim_{d\to 0} \frac{\Omega_1}{kd} = \cosh ky_0 - ky_0 \sinh ky_0 \\[2ex]
\lim_{d\to 0} \frac{\lambda_2}{kd} &= \sinh ky_0; \quad \lim_{d\to 0} \frac{\Omega_2}{kd} = \sinh ky_0 - ky_0 \cosh ky_0.
\end{aligned}\right\} \quad\text{..........} (24)$$

Then for a finite rectangle the solution is given by (23) when the values of (24) are used in (12) and (14).

In particular if $y_0 = 0$ and the line load is upon the x axis, the deflection surfaces are given by (23) in which the constants are

$$\left.\begin{aligned}
2A^{(1)}_n = 2A^{(2)}_n &= \frac{\Delta_4}{\Delta_1} - \frac{(\Delta_1\Delta_2 - \Delta_3\Delta_4)}{\Delta_1(1-\mu)} \\[2ex]
2B^{(1)}_n = -2B^{(2)}_n &= -1 \\
2C^{(1)}_n = -2C^{(2)}_n &= 1 \\[2ex]
2D^{(1)}_n = 2D^{(2)}_n &= \frac{-\Delta_3 - (1-\mu)}{\Delta_1}
\end{aligned}\right\} \quad\text{.......................} (25)$$

The symmetry is evident since W_1, for $y > 0$ is identical with W_2 for $y < 0$.

For an infinite plate strip, $y_0 = 0$ and the load is on the x axis, then equations (19) and (25) give

$$A^{(1)}{}_n = -B^{(1)}{}_n = C^{(1)}{}_n = -D^{(1)}{}_n = \tfrac{1}{2},$$

and the deflection[7] of the infinite strip is

$$W_1 = \frac{qa^3}{N\pi^4} \sum_1^\infty \frac{1}{n^4} (1 + ky) e^{-ky} \sin kx_0 \sin kc \sin kx \quad\text{..........................(26)}$$

Concentrated Loads. If c approaches zero in such a way that $2qc$ becomes a concentrated load P at the point (x_0, y_0), then it follows from (23) that

$$W_i = \frac{Pa^2}{N\pi^3} \sum_1^\infty \frac{1}{n^3} Y^{(i)}{}_n (y) \sin kx_0 \sin kx, \ (i = 1,2) \quad\text{...........................(27)}$$

in which the constants of (12) and (14) have the values:

$$
\left.
\begin{aligned}
&2A^{(1)}{}_n = 2A^{(2)}{}_n + 2\Omega_2 = \frac{-\lambda_1 (\Delta_1\Delta_2 - \Delta_3\Delta_4)}{\Delta_1 (1-\mu)} + \frac{\Delta_4\Omega_1}{\Delta_1} + \Omega_2 \\[2mm]
&2B^{(1)}{}_n = 2B^{(2)}{}_n - 2\Omega_1 = \frac{-\lambda_2 (\Delta_1\Delta_2 - \Delta_3\Delta_4)}{\Delta_2 (1-\mu)} - \frac{\Delta_3\Omega_2}{\Delta_2} - \Omega_1 \\[2mm]
&2C^{(1)}{}_n = 2C^{(2)}{}_n + 2\lambda_1 = \frac{1}{\Delta_2} \left\{ \lambda_2\Delta_4 + \lambda_1\Delta_2 - \Omega_2(1-\mu) \right\} \\[2mm]
&2D^{(1)}{}_n = 2D^{(2)}{}_n - 2\lambda_2 = \frac{1}{\Delta_1} \left\{ -\lambda_2\Delta_1 - \lambda_1\Delta_3 - \Omega_1 (1-\mu) \right\}
\end{aligned}
\right\} \text{.(28)}
$$

The limiting values of λ_1, λ_2, Ω_1, and Ω_2, already found in (24), are to be used here.

For a load on the central axis at $(x_0, 0)$, the complete solution for a finite rectangle is

$$W_1 = \frac{Pa^2}{2N\pi^3} \sum_1^\infty \frac{1}{n^3} \left[\cosh ky \left\{ \frac{(3+\mu)^2 - (1-k^2b^2)(1-\mu)^2 + 4(1-\mu)\Delta_4}{4\Delta_1(1-\mu)} + ky \right\} \right.$$

$$\left. - \sinh ky \left\{ 1 + ky\left(\frac{\Delta_3 + (1-\mu)}{\Delta_1}\right) \right\} \right] \sin kx_0 \sin kx. \quad\text{..............(29)}$$

For the case of a centrally loaded rectangular plate with two edges free, A. E. Love[8] gives a solution involving the singularity under the load and

[7] A. Nadai. l. c. p. 81.

[8] A. E. H. Love. Proc. Royal Soc. of London, Ser., A, 118: 427. (1928.)

certain biharmonic functions given by infinite series with coefficients some of which are expressed by infinite sums.

When $b \to \infty$ one obtains as a special case of (29)

$$W_1 = \frac{Pa^2}{2N\pi^3} \sum_1^\infty \frac{1}{n^3} \left[(1+ky)\cosh ky - (1+ky)\sinh ky \right] \sin kx_0 \sin kx$$

$$= \frac{Pa^2}{2N\pi^3} \sum_1^\infty \frac{1}{n^3} (1+ky)e^{-ky} \sin kx_0 \sin kx, \quad \text{.. (30)}$$

for the deflection[9] of an infinite plate strip due to a concentrated load at $(x_0, 0)$. This result may be verified directly from (26).

PINNED-CLAMPED PLATE

The problem of a rectangular plate which is pinned at two opposite edges and clamped at the other pair of edges, under the action of similar loading conditions as the previous case, is added since it may be obtained from the "pinned-free" plate by a device other than the usual super-position of an additional solution of the homogeneous plate equation.

At the clamped edges $2y = \pm b$, the conditions replacing (4) are

$$W_i \left(x, \pm \frac{b}{2} \right) = \frac{\partial W_i}{\partial y} = 0, \qquad (i = 1,2) \quad \text{.. (31)}$$

When the last four conditions of (8) are replaced by (31), it is noted that the constants of (12), (13) and (14) will not contain μ, while in the "pinned-free" plate the two combinations of $(3+\mu)$ and $(1-\mu)$ are due to conditions (4). A critical comparison of the forms resulting from operating on $Y^{(1)}_n$ and $Y^{(2)}_n$, of (6), by (31) with the corresponding ones resulting from (4), will show that if the expressions $(3+\mu)$ and $(1-\mu)$ are replaced by $+1$ and -1, respectively, the solutions for the "pinned-clamped" plate may be obtained from the problem considered earlier in this paper.

The constants, corresponding to (12), (13) and (14), for the "pinned-clamped" plate in the general case of a rectangular area of loading, are the following:

$$2A^{(1)}_n - \Omega_2 = 2A^{(0)}_n + \Omega_3 = 2A^{(2)}_n + \Omega_2 = \frac{2\Omega_1 \sinh^2 a - 2\lambda_1 a^2}{2a + \sinh 2a}$$

$$2B^{(1)}_n + \Omega_1 = 2B^{(0)}_n - \Omega_4 = 2B^{(2)}_n - \Omega_1 = \frac{2\Omega_2 \cosh^2 a + 2\lambda_2 a^2}{2a - \sinh 2a}$$

$$2C^{(1)}_n - \lambda_1 = 2C^{(0)}_n + \lambda_4 = 2C^{(2)}_n + \lambda_1 = \frac{-2\Omega_2 - 2\lambda_2 \sinh^2 a}{2a - \sinh 2a}$$

$$2D^{(1)}_n + \lambda_2 = 2D^{(0)}_n - \lambda_3 = 2D^{(2)}_n - \lambda_2 = \frac{2\Omega_1 - 2\lambda_1 \cosh^2 a}{2a + \sinh 2a}$$

.... (32)

---

[9] A. Nadai. l. c.

in which $2a = kb$.

The explicit form for W_1 is expressed by

$$W_1 = \frac{4pa^4}{N\pi^5} \sum_1^\infty \frac{1}{n^5} \sin kx_0 \sin kc \sin kx \left[\cosh ky \left\{ \frac{\Omega_1 \sinh^2 a - \lambda_1 a^2}{2a + \sinh 2a} + \frac{\Omega_2}{2} \right\} \right.$$

$$+ \sinh ky \left\{ \frac{\Omega_2 \cosh^2 a + \lambda_2 a^2}{2a - \sinh 2a} - \frac{\Omega_1}{2} \right\} + ky \cosh ky \left\{ \frac{\lambda_1}{2} - \frac{\Omega_2 + \lambda_2 \sinh^2 a}{2a - \sinh 2a} \right\}$$

$$\left. + ky \sinh ky \left\{ \frac{\Omega_1 - \lambda_1 \cosh^2 a}{2a + \sinh 2a} - \frac{\lambda_2}{2} \right\} \right] . \qquad \left(y_0 + d \leqq y \leqq \frac{b}{2} \right) \dots\dots (33)$$

Equations for W_0 and W_2 similar to (33) may be obtained from (5) and (6) by inserting the appropriate constants from (32). These equations confirm the results of H. Schmidt[10] obtained for the same type of loading and the same boundary conditions.

The solution for a uniformly loaded plate is

$$W_0 = \frac{4pa^4}{N\pi^5} \sum \frac{1}{n^5} \left[1 - \left(\frac{2 \sinh a + 2a \cosh a}{2a + \sinh 2a} \right) \cosh ky \right.$$

$$\left. + \left(\frac{2ky \sinh a}{2a + \sinh 2a} \right) \sinh ky \right] \sin kx , \dots\dots\dots\dots (34)$$

in which $n = 1, 3, 5, \dots\dots$.

The solution for a concentrated load at $(x_0, 0)$, is

$$W_1 = \frac{Pa^2}{2N\pi^3} \sum_1^\infty \frac{1}{n^3} \left[\frac{(2 \sinh^2 a - 2a^2) \cosh ky - 2 ky \sinh^2 a \sinh ky}{2a + \sinh 2a} \right.$$

$$\left. - \sinh ky + ky \cosh ky \right] \sin kx_0 \sin kx, \qquad \left(k = \frac{n\pi}{a} \right) \dots\dots (35)$$

where $2a = kb$. This yields known solutions [11] for the centrally loaded pinned-clamped plate.

The results of (34) and (35) may be obtained directly from the explicit forms of (18) and (29) by the replacements mentioned. Any further cases will follow from equations similar to (33), since these do not contain μ.

---

[10] H. Schmidt. Zeitschrift f. Ang. Math. und Mechanik, Band 12, Heft 3, S. 142.

[11] S. Timoschenko. Der Bauingenieur, Heft 2, S. 51, 1922. H. M. Westergaard. Public Roads, Journal Highway Research, U. S. Dept. of Agriculture, 11, (No. 1) 1930, p. 20.

CONCLUSION

Using a lemma of Levy, the author obtains the deflection of a rectangular plate uniformly loaded over a portion of the plate which is bounded by lines parallel to the edges of the plate. For a plate with two opposite edges simply supported and two edges free, the solution is readily obtained if the plate is divided into three strips and the necessary conditions are satisfied at the lines of division. By specialization, the important cases of uniform loading, line loading, and concentrated loads are derived for the finite rectangle and for the infinite plate strip. A critical study of the boundary conditions enables one to derive the case of two edges simply supported and two edges clamped from the previous case. All the special cases check with results previously obtained by other writers.

THE 1934 SPRING MIGRATION OF SHORE BIRDS THROUGH CLAY AND PALO ALTO COUNTIES, IOWA[1]

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These migration data on shore birds were taken while studying and making observations on migratory waterfowl in northwest Iowa from March 12 to July 28. As the shore bird data for this part of Iowa were limited, much time was spent in the field every day checking the arrivals and numbers of the respective species.

When there was any doubt as to the identification of a bird, collecting was done. All specimens taken are now in permanent possession of the Zoology Department, Iowa State College, Ames, Iowa.

The following graphs (figs. 1-4) give complete populations on dates recorded from the time each species was first seen until the flight was over. The discussion under each species gives a short summary about the type of habitat in which it was observed.

PIPING PLOVER
Charadrius melodus Ord. (Fig. 1)
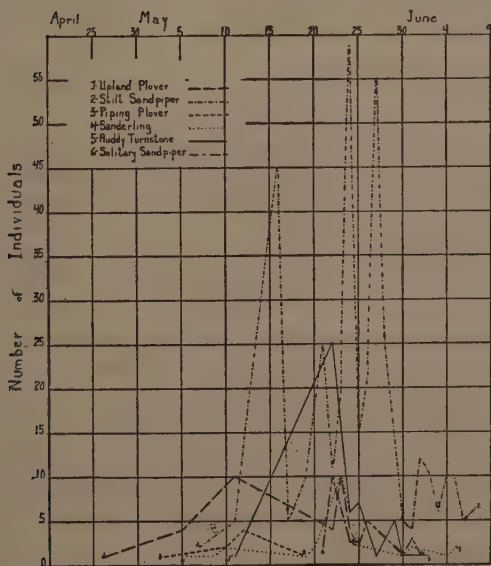
Fig. 1. Spring flight of Upland Plover, Stilt Sandpiper, Piping Plover, Sanderling, Ruddy Turnstone and Solitary Sandpiper.

Between the dates of May 3 and May 19, eleven birds of this species were seen. Eight of these birds were on the sandy beaches of Lost Island Lake in Clay County; three on the mud flats of a small pond in Palo Alto County. One bird was collected May 3, Lost Island Lake, Clay County.

SEMIPALMATED PLOVER
Charadrius semipalmatus Bonaparte (Fig. 3)

One of the common migrants through Clay and Palo Alto Counties. The first arrivals were noted May 2. They were seen from that time on until June 5. In practically all cases they were observed on sandy beaches.

KILDEER
Oxyechus vociferus vociferus (Linnaeus)

On March 16 five kil-

deers were seen on Lost Island Lake, Clay County, the first shore birds of the season. From that time on they were seen until late in April. The killdeer seemed to be the most versatile of all our shore birds as to feeding and wading places chosen. One would see them on sandy beaches, mud flats, drainage ditches, and in stubble fields. It would be rather difficult to determine the last date of spring departure as they are common summer residents throughout the state.

AMERICAN GOLDEN PLOVER

Pluvialis dominica dominica (Müller) (Fig. 3)

This species is a rather scarce migrant. Eleven birds were seen May 7 on mud flats near Lost Island Lake in Clay County. Two were observed on a rocky reef in Lost Island Lake, Clay County, May 17. A single bird was recorded on May 28 for Round Lake, Clay County.

BLACK-BELLIED PLOVER

Squatarola squatarola (Linnaeus) (Fig. 3)

This bird was seen a number of times from April 27 to June 1. Seventeen, the largest number of birds of this species seen at one time, were observed on a sandy point in Trumbull Lake, Clay County, May 28. One specimen was collected on Lost Island Lake, Palo Alto County, May 22. At all times these birds were on rock or sandy beaches.

RUDDY TURNSTONE

Arenaria interpres morinella (Linnaeus) (Fig. 1)

Fifty-two birds of this species were seen from May 11 to June 2; twenty-five were observed on May 22 in Clay and Palo Alto Counties. Many were seen running in and about the bullrushes and other sedges back from the water's edge.

WILSON'S SNIPE

Capella delicata (Ord.) (Fig. 3)

A common migrant through northwest Iowa and may be seen on practically all of the sloughs and marshes from April 22 to May 16.

UPLAND PLOVER

Bartramia longicauda (Bechstein) (Fig. 1)

A rather common migrant and nesting species in Clay and Palo Alto Counties. These birds were seen from May 5 throughout the summer in the meadows and pastures.

SPOTTED SANDPIPER

Actitis macularia (Linnaeus) (Fig. 3)

A common migrant and nesting bird, first observed May 1.

EASTERN SOLITARY SANDPIPER

Tringa solitaria solitaria Wilson (Fig. 1)

Fairly numerous from May 21 to June 1. This bird was seen more often in wet places overgrown with vegetation, such as sloughs and marshes.

WESTERN WILLET

Catoptrophorus semipalmatus inornatus (Brewster)

An uncommon migrant. The following were recorded for this area. One, May 1 (collected), Lost Island Lake, Clay County; two, May 7, Lost Island Lake, Clay County; two, May 11, Lost Island Lake, Palo Alto County; and one, May 16, Lost Island Lake, Palo Atlo County. All birds were seen feeding in clear, shallow water.

GREATER YELLOWLEGS

Totanus melanoleucus (Gmelin) (Fig. 2)

A moderate continuous flight took place from April 6 to May 5. These birds were seen in almost any sort of wet habitat.

LESSER YELLOWLEGS

Totanus flavipes (Gmelin) (Fig. 4)

A very common migrant. Seen from April 8 to June 3. Like the Greater Yellowlegs, this bird seemed to frequent all sorts of wet places.

AMERICAN KNOT

Calidris canutus rufus (Wilson)

Dr. Paul L. Errington and the writer observed fourteen of these birds May 21 on the rocky shore of Lost Island Lake, Palo Alto County. The birds were observed at close range through ten power binoculars. The bright red underparts, comparatively short bill, and upon flushing, their close, compact flight formation, left no doubt as to their identification.

PECTORAL SANDPIPER

Pisobia melanotos (Vieillot) (Fig. 2)

Hundreds of these birds migrated through this region. They were observed from April 2 to June 2. They were seen in pastures, mud flats, sandy beaches, and along marshy shore lines. Especially after rains they could be seen scurrying and flying over the pastures and meadows.

WHITE-RUMPED SANDPIPER

Pisobia fuscicollis (Vieillot) (Fig. 2)

One of our most common migrants. Many were seen from May 12 to June 3. Most of them were observed wading and running about on sandy shores in shallow water.

BAIRD'S SANDPIPER
Pisobia bairdi (Coues) (Fig. 4)

A fairly common migrant in Clay and Palo Alto Counties. About two hundred and fifty were recorded between May 10 and May 23. All were seen frequenting the clear, sandy beaches.

LEAST SANDPIPER
Pisobia minutilla (Vieillot) (Fig. 4)

An abundant migrant. Hundreds passed through northwest Iowa this spring. A continuous flight from April 19 to June 1 was noted. These little sandpipers seemed to prefer the water's edge of the sandy and rock strewn beaches. The most observed on one date was one hundred and twenty-five in Clay and Palo Alto Counties on May 22.

RED-BACKED SANDPIPER
Pelidna alpina sakhalina (Vieillot) (Fig. 4)

Over one thousand red-backed sandpipers were seen from May 7 to June 7. Flocks of thirty and forty were seen feeding in the shallow water of Lost Island Lake in Clay and Palo Alto Counties from May 22 to May 25.
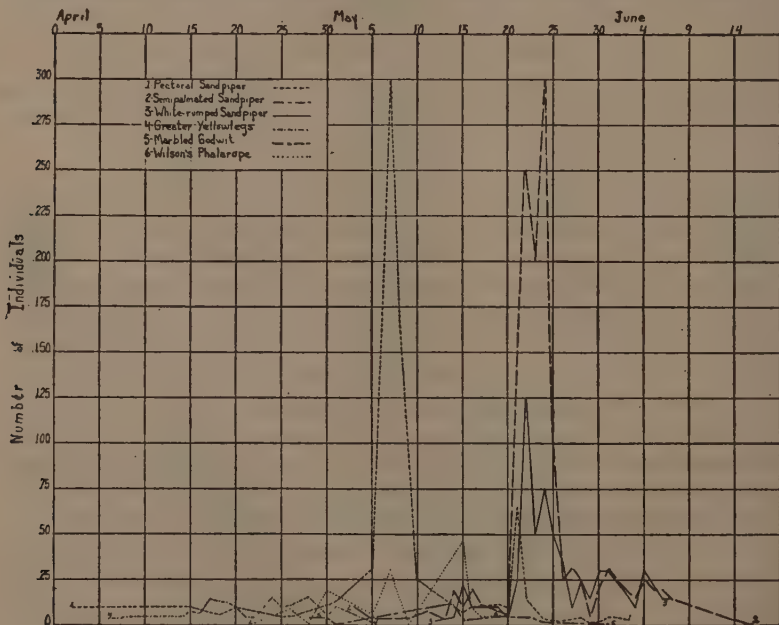
Fig. 2. Spring flight of Pectoral Sandpiper, Semipalmated Sandpiper, White-rumped Sandpiper, Greater Yellowlegs, Marbled Godwit and Wilson's Phalarope.

DOWITCHER

Limnodromus griseus subsp. (Fig. 3)

One hundred and thirty-three of these birds were recorded from May 10 to May 22. How many were Eastern or Long-billed, the writer cannot say. Two birds collected on May 16 were identified as Eastern Dowitchers. They were seen in both Clay and Palo Alto Counties.

STILT SANDPIPER

Micropalma himantopus (Bonaparte) (Fig. 1)

A common migrant in Clay and Palo Alto Counties. These birds were observed from May 7 to June 7. Several hundred were seen in this area. They seemed to occur in about even numbers on mud and sand flats.

SEMIPALMATED SANDPIPER

Ereunetes pusillus (Linnaeus) (Fig. 2)

A very common migrant through this area. Over one thousand were seen from May 3 to June 3. Most of them were observed wading in shallow waters with sandy bottoms and beaches.

WESTERN SANDPIPER

Ereunetes maurii Cabanis

A rare spring migrant. Three were seen on the sand beaches of Lost Island Lake, Palo Atlo County, May 22.
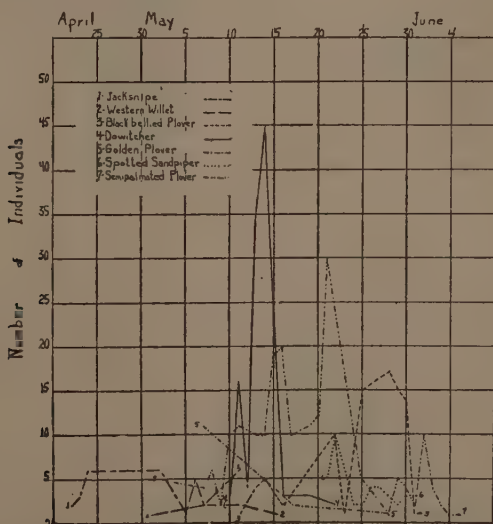
Fig. 3. Spring flight of Jacksnipe, Western Willet, Black-bellied Plover, Dowitcher, Golden Plover and Spotted Sandpiper.

MARBLED GODWIT

Limosa fedoa (Linnaeus) (Fig. 2)

Forty-one of these large shore birds were seen in Clay and Palo Alto Counties between April 28 and June 2. One bird was collected in a reed grown marsh near Trumbull Lake, Clay County, May 1. However, most of the birds observed were in shallow waters adjoining sandy beaches.

HUDSONIAN GODWIT

Limosa haemastica (Linnaeus) (Fig. 4)

Eighty-one of these beautiful birds were observed from April 13 to May 26. On May 13 and 14 a flock of ten birds was observed on Lost Island Lake in Clay and Palo Alto Counties. These birds were seen feeding in both sand and mud bottomed waters. Philip A. DuMont and the writer observed the first one of the season April 13, Lost Island Lake, Clay County. This is apparently a new early spring record for the United States, according to migration dates given by Bent (1927).

SANDERLING

Crocethia alba (Pallas) (Fig. 1)

Not a great many of these birds were observed. Twenty-eight indi-
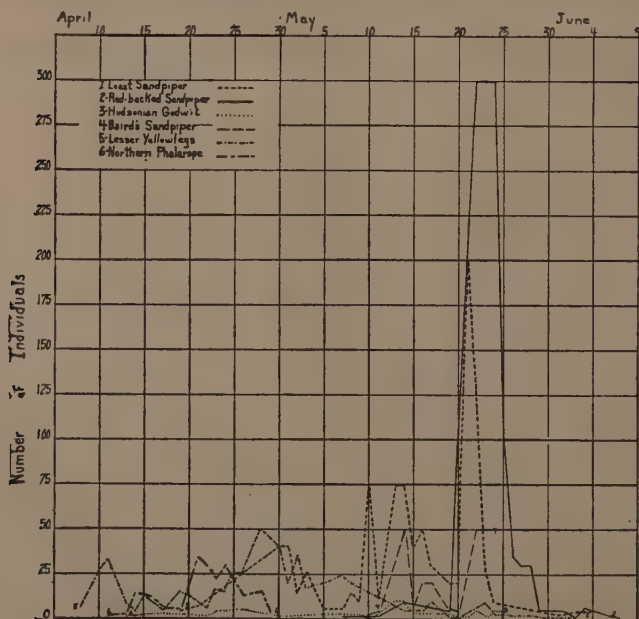
Fig. 4. Spring flight of Least Sandpiper, Red-backed Sandpiper, Hudsonian Godwit, Baird's Sandpiper, Lesser Yellowlegs and Northern Phalarope.

viduals were seen from May 5 to June 5. All birds were seen running along the sandy beaches of Lost Island Lake in Clay and Palo Alto Counties.

AVOCET

Recurvirostra americana Gmelin

A lone Avocet was seen on Lost Island Lake, Clay County, May 13. The bird was flushed and it flew across the lake to the Palo Alto side. Presumably the same bird was collected May 14 on the Clay County side of Lost Island Lake. Another was seen flying over Trumbull Lake, Clay County, on June 28.

WILSON'S PHALAROPE

Steganopus tricolor Viellot (Fig. 2)

One hundred and forty-four birds were recorded from April 22 to May 24 for Clay and Palo Alto Counties. Most of the birds were observed feeding in sand bottomed shallow waters, but a few were seen in mud bottomed ponds.

NORTHERN PHALAROPE

Lobipes lobatus (Linnaeus) (Fig. 4)

A fairly common migrant. About two hundred and twenty-five were seen from May 11 to May 29. All birds were seen in clear, sand bottomed shallow waters. About equal numbers were noted for Clay and Palo Alto Counties.

The 1934 spring flight of shore birds compared with that of 1933 (Bennett, '34) shows a great variation in species and numbers recorded. Although my 1933 notes were incomplete, the differences are among the most conspicuous species of these birds. As the same area was observed in 1933 and 1934 the writer is assured that the 1934 spring flight was radically different from that of the preceding year. The following comparisons are examples of a few of the outstanding variations:

	Birds Observed	
	1933	1934
Avocet	0	2
Hudsonian Godwit	3	81
Marbled Godwit	0	41
Red-backed Sandpiper	3	1000 or more
Ruddy Turnstone	0	52
Western Willet	0	6
Piping Plover	0	11
Dowitcher	0	133
Stilt Sandpiper	6	300 or more
American Knot	0	14

There appear to be two main reasons for such a difference in spring flights: That certain species may or may not traverse the same route year after year; or that the continued drought in the west, resulting in

the drying up of a large percentage of shore bird habitats, may have forced the birds through areas where the lake levels are near normal, as they are in northwest Iowa.

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THE 1934 FALL MIGRATION OF SHORE BIRDS THROUGH CLAY AND PALO ALTO COUNTIES, IOWA[1]

Gerald B. Spawn

From the Entomology and Economic Zoology Section, Iowa Agricultural Experiment Station

The data presented in the following paragraphs were collected in Clay and Palo Alto Counties, in northwest Iowa, from June 25 to November 17, 1934. The studies were conducted mainly on the shores of Lost Island Lake. Observations on certain species, however, were necessarily made in other localities due to differences in habitat preferences. These localities were all within five miles of the lake.

Lost Island Lake is situated on the boundary line between Clay and Palo Alto Counties. Two particular areas along the shores of the lake were used most extensively by the shore birds. One of these, a strip of beach about a quarter of a mile in length, is located in Clay County; the other, which includes about one-half mile of the shore line, is in Palo Alto County. The majority of these data deal collectively with these two shores. Observations on these two areas alone are the basis for the construction of all the graphs except that for the Killdeer. Casual observations elsewhere were disregarded.

The graphs were constructed on the basis of average numbers of birds seen on the two shores over five-day periods. To obtain the points on the graphs the daily observations for the two areas were averaged. The five-day averages were computed from these daily averages. The five-day periods were started on June 25. That date was considered to be the first record of the season for a migrating shore bird. Consequently the first five-day period was for June 25-29, inclusive. In the event of observations having been taken on less than five days of any one period the average was based on the actual number of days upon which observations were made.

A number of specimens were collected. These were taken for two reasons: verification of field identifications, and furtherance of subsequent studies under the supervision of the Zoology Department of Iowa State College. The prepared specimens are now in permanent possession of this department.

For records and notes on shore birds in the Lost Island Lake vicinity from June 25 to July 17, the author wishes to acknowledge his indebtedness to Mr. Logan J. Bennett.

This paper deals with observations on twenty-five species of birds. The discussions of the various species appear in sequence according to their order in the American Ornithologists' Union check-list.

PIPING PLOVER

Charadrius melodus Ord.

Three of these birds were observed by Mr. Logan J. Bennett and the author on August 10. They were seen on the rather flat, sandy beaches of Lost Island Lake in Palo Alto County. Two were collected. This species is "a rare migrant and a casual summer resident—probably breeding in Iowa" (3).

SEMIPALMATED PLOVER

Charadrius semipalmatus Bonaparte

Four hundred eighty-five birds were seen from July 18 to October 6, all observed on the beaches of Lost Island Lake. The migration peak came between August 29 and September 3 (Graph, fig. 1). It is considered a fairly common migrant.

KILLDEER

Oxyechus vociferus vociferus (Linnaeus)

A common summer resident throughout Iowa. In view of this fact it is rather difficult to say just when the fall migration started. The graph (fig. 1) indicates that the peak of migration occurred between September 12 and 22. They were observed in a variety of habitats, mainly, however, around the shores of Lost Island Lake, mud-bottomed ponds, and in grazed pastures. This was one of the last two species of the season to be seen.

AMERICAN GOLDEN PLOVER

Pluvialis dominica dominica (Müller)

An uncommon migrant. Two were seen and collected November 11 on Lost Island Lake, Clay County.

BLACK-BELLIED PLOVER

Squatarola squatarola (Linnaeus)

This species appeared to be rare as a fall migrant through the Lost Island Lake area this year. A single bird was observed and collected on Lost Island Lake, Clay County, August 10.

RUDDY TURNSTONE

Arenaria interpres morinella (Linnaeus)

In contrast to Benentt's (1) record of 52 in the spring, only four were seen this fall. They were observed on the shores of Lost Island Lake and were turning over small, flat stones supposedly to feed on the small animal life thus exposed. One was seen August 3 in Clay County; two on August 15 and one on September 19 in Palo Alto County. This species is a rare migrant.

AMERICAN WOODCOCK

Philohela minor (Gmelin)

One bird was observed by Mr. Logan J. Bennett and the author on Mud Lake, Clay County, August 7. DuMont (3) considers this species "a decidedly uncommon migrant and a rare summer resident in the eastern half of the state."

WILSON'S SNIPE

Capella delicata (Ord.)

The first Snipe was observed on September 6. From this date up to October 7, 39 were recorded. On October 21 a more or less typical Snipe habitat was found at Whitford Slough, two and one-half miles west of Ruthven, Clay County. This area, which is about 10-15 acres in extent, each year supports generous growths of sedges, bulrushes, and other marsh plants. In the fall of 1934 there was from three to nine inches of water over most of the marsh. On October 21, 23 Snipe were seen in this area. Five subsequent observations gave results as follows: Oct. 27, 25; Oct. 28, 30; Nov. 3, 40; Nov. 10, 25; Nov. 17, 12. Field observations were discontinued on Nov. 17. It is quite probable, however, that Snipe could have been found for a few days after that date.

SPOTTED SANDPIPER

Actitis macularia (Linnaeus)

This bird was found breeding on the Palo Alto County shore of Lost Island Lake. Upon several occasions downy young were observed running along the shore. One of these was collected and preserved. The author is confident that quite a number of those observed were resident rather than migratory birds. This, however, should not appreciably affect the migration peak as shown by the accompanying graph (fig. 1) inasmuch as the daily percentage of error due to resident birds should be practically constant, at least until the resident birds themselves migrate. The peak of migration apparently came about the middle of August. The last record of observation was of one bird on September 12. DuMont (3) considers this bird "a common migrant and fairly numerous summer resident, breeding throughout the state."

EASTERN SOLITARY SANDPIPER

Tringa solitaria solitaria Wilson

"A fairly common migrant along all of the rivers and streams of the state" (3). In the areas under observation in Clay and Palo Alto Counties this fall they were moderately common. Twenty-nine birds were seen between July 5 and September 12. They were found mainly around ponds with mud or a mixture of sand and mud bottoms.

GREATER YELLOW-LEGS

Totanus melanoleucus (Gmelin)

This bird is ordinarily "a fairly common spring and fall migrant, fluctuating somewhat in numbers and seldom as numerous as the Lesser

Yellow-legs" (3). Fourteen birds were seen between July 20 and September 6 on Lost Island Lake, Clay and Palo Alto Counties.

LESSER YELLOW-LEGS

Totanus flavipes (Gmelin)

With regard to the total number of individuals seen this species ranks second only to the Least Sandpiper. Observations totalling 1,283 birds were recorded from June 25 to October 20. The heaviest flight came between August 13 and September 12 (Graph, fig. 1). The data seem to show no definite peak of migration but rather three periods of noticeably heavier flight. With the exception of occasional casual observations in other localities, which were not considered in construction of the graph, these birds were all seen around the shores of Lost Island Lake. They were seen most often feeding in water from "tarsus-deep" to "breast-deep". Some were seen around mud-bottomed ponds.

PECTORAL SANDPIPER

Pisobia melanotos (Vieillot)

A common migrant through this area. Eleven hundred and forty-three birds were seen from July 14 to October 27. The largest flight came between July 27 and August 23 with the peak between July 28 and August 4 (Graph, fig. 1). With a few exceptions these birds were seen on the sandy shores of Lost Island Lake. On five occasions groups of 2, 5, 6, 10 and 12, respectively, were observed feeding around mud-bottomed pasture and barnyard ponds.

WHITE-RUMPED SANDPIPER

Pisobia fuscicollis (Vieillot)

Only one individual of this generally common species was observed. It was in company with eight Pectoral Sandpipers which were feeding in the shallow water along the flat, sandy shores of Lost Island Lake, Clay County, August 28.

BAIRD'S SANDPIPER

Pisobia bairdi (Coues)

Eighty-four birds were seen between August 21 and September 29. There was a more or less continuous flight between these two dates (fig. 1). This species is "a fairly common spring migrant, less numerous in the fall" (3).

LEAST SANDPIPER

Pisobia minutilla (Vieillot)

The Least Sandpiper occurred in larger numbers than any other species seen during this series of observations. A total of 1,829 birds was seen between July 10 and October 27. The heaviest flight came between August 19 and September 12 with the peak of migration between August 24 and 28 (Graph, fig. 1). By far the majority of the birds were observed

feeding in the shallow water and on the wave-washed shores of Lost Island Lake, Clay and Palo Alto Counties. Some were seen feeding on the sand flats as far as 40 or 50 feet from the water. During the late forenoon and early afternoon they spent much time preening their feathers and loafing in the sun among the small rocks which are found in large numbers at several points along the beach. On several occasions small groups were seen feeding around mud-bottomed ponds in pastures and barnyards.
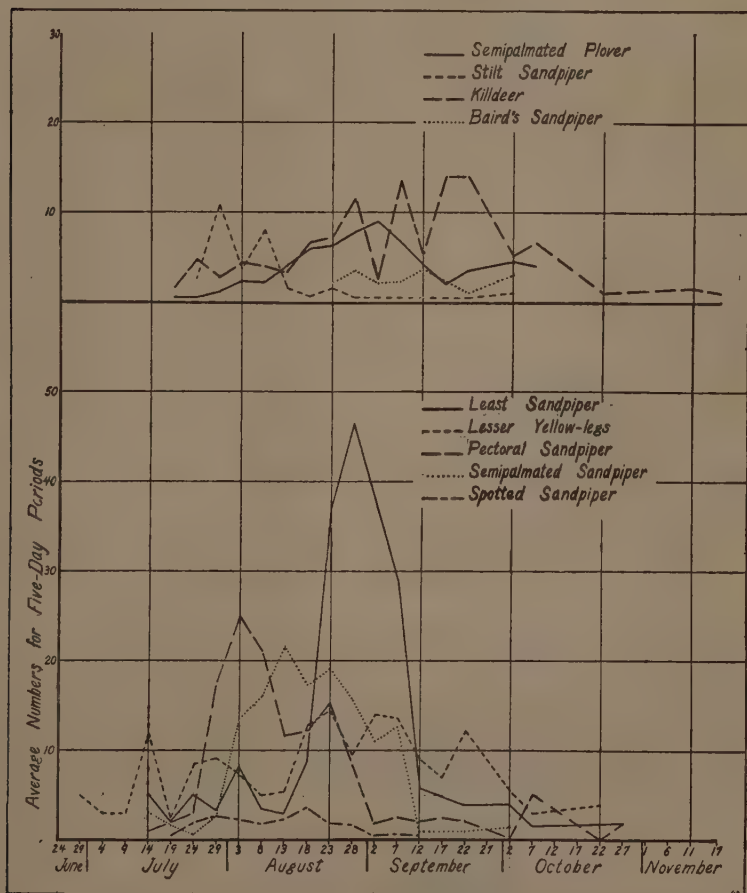
Fig. 1. Graphs showing average numbers of shore birds seen at two observational areas on the shores of Lost Island Lake, Clay and Palo Alto Counties, Iowa. The graphs are based on average numbers of birds seen over consecutive five-day periods from June 25 to Nov. 17, 1934.

RED-BACKED SANDPIPER

Pelidna alpina sakhalina (Vieillot)

In contrast with Benentt's (1) record of over a thousand observed during the spring migration, a single bird was seen and collected on the short of Lost Island Lake, Clay County, October 27.

DOWITCHER

Eastern—*Limnodromus griseus griseus* (Gmelin)

Long-billed—*Limnodromus griseus scolopaceus* (Say)

Due to the probable inability to properly identify the two species in the field Eastern and Long-billed Dowitchers are discussed as one species in this paper. However, of the 33 birds observed, the author believes that 11 were the Long-billed and 22 the Eastern Dowitcher. The first record was of two on July 10 and the last record was of one on October 20. There was no definite peak of migration. They were seen on the sand beaches of Lost Island Lake and around mud-bottomed ponds. Of six specimens collected and prepared by the author the lengths, in millimeters, of the exposed culmens were as follows: 56, 58, 58, 60, 70 and 78. Eastern and Long-billed Dowitchers are considered by DuMont (3) to be rare and fairly rare migrants, respectively.

STILT SANDPIPER

Micropalama himantopus (Bonaparte)

The first fall record was of five birds on July 23 and the last was of two on September 29. Between these two dates 121 were seen. The peak of migration came between July 27 and August 4 (fig. 1). They were rather common migrants through northwestern Iowa this fall. Eleven were collected.

SEMIPALMATED SANDPIPER

Ereunetes pusillus (Linnaeus)

This species was one of the most common migrants through the area under observation. Twelve hundred eight birds were seen between July 10 and September 29. The heaviest migration took place from August 1 to September 2 (Graph, fig. 1) with the peak falling between August 8 and 13. With but a single exception these birds were all seen on the shores of Lost Island Lake, Clay and Palo Alto Counties. They seem to prefer to feed in shallow water as do Least Sandpipers, but unlike the latter they are less frequently seen feeding farther than five feet from the water's edge.

WESTERN SANDPIPER

Ereunetes maurii Cabanis

Eighteen birds were seen between July 9 and August 25. One was collected. Those observed were in company with Least and Semipalmated Sandpipers. DuMont (3) states that "the present status of these birds in Iowa is undetermined." My observations would indicate that this

bird is a moderately common late summer migrant through northwestern Iowa. With regard to this and certain other species which have appeared in numbers somewhat larger than usual, the status indicated by the past season's study may be influenced somewhat by drought conditions in the country as a whole.

BUFF-BREASTED SANDPIPER

Tryngites subruficollis (Vieillot)

A rare spring and fall migrant (3). Roberts (4) considers this species of such rare or accidental occurrence that they are not likely to be seen. Two birds were seen August 26 on Lost Island Lake, Palo Alto County. On the following day one was seen on the Palo Alto County shore and one on the Clay County shore. It seems probable that the same birds were seen on the two days. None of these were collected. However, the writer is familiar with this bird through having recently collected one in South Dakota. This specimen is preserved and in permanent possession of the Zoology Department, Iowa State College, Ames, Iowa.

SANDERLING

Crocethia alba (Pallas)

Between August 21 and October 6, 68 birds were seen. The apparent peak of migration came between August 25 and 31. These birds were all seen running along the beaches of Lost Island Lake. The Sanderling is considered a rare migrant (3); however, the records of the past season's study would seem to indicate that it is somewhat common.

WILSON'S PHALAROPE

Steganopus tricolor Vieillot

"A fairly common migrant. Formerly a common summer resident, breeding in the northern part of the state" (3). Observations indicate that it was rare in this area this fall. A single bird was seen and collected on Lost Island Lake, Clay County, September 3.

NORTHERN PHALAROPE

Lobipes lobatus (Linnaeus)

Three of these birds were seen on the shores of Lost Island Lake, Clay County, August 26. Dr. Paul L. Errington and the author observed and collected one on Lost Island Lake, Palo Alto County, August 30. DuMont (3) considers it "an uncommon migrant along the Missouri River Valley, rare in other parts of the state." Bennett (1) found it a fairly common migrant in the spring of 1934. It was apparently rare in northwestern Iowa in the fall of 1934.

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BOB-WHITE WINTER SURVIVAL ON EXPERIMENTALLY SHOT AND UNSHOT AREAS[1]

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Iowa bob-whites (*Colinus virginianus virginianus* Linn) were in 1916 given the legal protection of a completely closed season which continued up to the autumn of 1933. For experimental purposes, however, the Iowa Fish and Game Commission, in cooperation with Iowa State College, permitted some carefully controlled shooting in late November and early December, 1933, on fourteen selected official game management areas.

One of the primary purposes of the shooting (for report, see Schuenke, 1933) and subsequent studies was to test further the population vulnerability thesis derived from four years previous field work in north-central states (Errington, 1934).

The population vulnerability thesis pertaining to bob-white holds that severe winter predation upon adult vigorous birds is restricted to that part of the population in excess of what may be called the normal carrying capacity of the land, or the maximum number that the land could winter under the most favorable conditions. If more birds are present than the environment at its best can accommodate, obviously the extra or surplus birds either have to leave or be killed. These extra birds, ill-situated in their environment, bear the brunt of predator attacks.

On the other hand, if the winter quail population is fit and within the normal carrying capacity of the land—whether population or carrying capacity be high or low—its vulnerability to predators is low and it is subject to slight losses, aside from those brought on as by climatic emergencies or by shooting.

The severity of simple predation, then, upon winter bob-white has seemed to resolve itself into a matter of how much the environment was over-populated. This thesis has been presented with observational evidence in considerable detail elsewhere (Errington, 1934, and introduced more briefly by Errington, 1933b; 1933d).

Normal winter carrying capacities of Iowa and Wisconsin observational areas, on the basis of evidence in possession, were determined by the quality and distribution of coverts habitable for quail. The habitability of these coverts was dependent upon the effectiveness of food and cover combinations.

The net amount of winter predation suffered by the quail did not seem correlated with the kinds and number of predators within the limits observed.

Seemingly irrespective of the composition of the wild predator population, quail populations top-heavy for an environment were vulnerable and lost at rates tending to eliminate the excess; and populations within

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environmental carrying capacity at the beginning of the winter remained quite securely entrenched against all enemies except for more efficient man.

Carrying capacities, as measured by maximum spring survivals over a period of years, showed in most cases a remarkable degree of constancy for specific areas.

With the rather voluminous Iowa and Wisconsin observational data as a background, significant questions arise as to what might be done by experimental manipulation of quail populations. Would, for example, the artificial removal of the vulnerable percentage, or the surplus population, actually give the survivors a materially increased winter security?

It might be in order, before examining the data by areas, to describe briefly the study technique used and the salient characteristics of the winter.

The winter was generally mild and snowless except for a few periods of severe weather. The openness of the winter was all that averted heavy starvation mortality, for the supply of quail food on most of the observational areas was extremely limited. Chinch bugs and drouth in southeastern Iowa, where most of the experimental shooting was done, seriously damaged both small grains and corn; the farmers, pressed for feed, harvested cleanly and then turned stock into the fields. The crop of pigeon grass, lesser ragweed and other weeds bearing important quail foods also was short. Further complicating the situation, the chinch bug infestation, combined with the dryness of the season, precipitated wholesale burning, which evicted countless coveys from their established territories.

Winter feeding was not conducted on a scale sufficient to offset materially the food shortage. With a few commendable exceptions, farmers and sportsmen alike, even when obligated by agreements on game management areas, neglected to provide adequate food when it was needed, to the consequent detriment of the quail.

Lack of snow and the unusual shifting of bob-white population in response to burning and on account of the failure of food sources made difficult efforts to acquire really reliable data on survival. Then, too, the large total acreage under observation necessitated less frequent visiting and on the whole less thorough work for the majority of areas.

The chief reliance was placed, where possible, upon a direct enumeration census technique, a modification of that which proved highly satisfactory in the Wisconsin studies (Errington, 1933a). The Wisconsin censuses, however, were made with the aid of more snow than we have had in Iowa and dealt with quail densities that were lower and hence more satisfactorily handled. With respect to this latter point, the likelihood of error in bob-white census work increases so conspicuously with increasing density that we do not feel at all sure of census figures on populations exceeding about a bird per four acres.

In many instances, the figures obtained by flushing and counting birds had to be supplemented by figures calculated on the basis of additional occupied territories, for which ample "sign" (droppings, dust baths, tracks and feathers) was to be found.

On three areas where the pressure of time did not allow even territorial calculations according to "sign," estimates of carrying capacity were made from the road by Errington, later to be checked by actual census

work. The checking was entirely independent of the original estimates, but the resulting figures were quite comparable.

For a two-hundred-acre portion of area "B" three covey territories were estimated on November 18; the check-up March 6 gave three coveys averaging thirteen birds.

Four covey territories were estimated on an unexamined sample of area "G" in early November, which a check on December 15 showed to be correct. The November estimate of these four territories, at the 13.5 birds per covey known average for the area as a whole, gave fifty-four birds; the December 15 check, after the shooting, at a 9.5 bird per covey average, gave thirty-eight, which, in view of the moderate shooting on this area, is about the number to be expected.

The population of a part of area "A" was estimated, on November 18, at one hundred and sixty-three birds in ten-covey territories; the January 5 census, after the shooting, showed nine occupied territories with about ninety-nine birds.

Thus, of the seventeen covey territories experimentally estimated without actual examination of the land, sixteen proved upon checking to be correct.

From the experiments indicated in the preceding paragraphs, it may be seen that experienced observers can often get a fairly accurate idea of a probable quail population merely by looking over the ground, always provided that the population is up to carrying capacity.

Estimates are not recommended as a substitute for the much more desirable territory calculations on the basis of "sign," nor for truly accurate censuses, except when vast acreages have to be surveyed hastily.

Advantageous use was made of dogs in census work on many areas, but dogs or no dogs, the skill and diligence of the field man appeared to be the main requisite for the securing of accurate counts.

Reports of hunters or farmers proved untrustworthy as a rule, in view of the almost invariable tendency of the public to over-estimate the numbers of birds.

Data from the shot areas and those from the unshot checks will be summarized as in possession, irrespective of completeness.

SHOT AREAS

Area "A"—840 acres. Sec's. 3, 4, 9, 10, Douglas township, Appanoose county. Pre-shooting check by Errington, Mr. and Mrs. Hamerstrom, Wardens C. H. Updegraff and J. C. Graham, November 18, 1933. Forty-nine flushed in 3 coveys; 23 estimated territories at 16.33 birds each gave 375 more. Total arrived at, 425, or a bird per 2 acres.

Experimental shooting November 23, 25, 28, 30, December 2—Warden J. W. O'Hara in charge. Ninety birds bagged; 34 reported crippled or lost. Total known toll from shooting, 124.

Post-shooting check by Wardens C. H. Updegraff and J. C. Graham, January 5, 8, 1934. Two hundred and fourteen flushed in 18 coveys; 5 calculated territories at 11.88 birds each gave 59 more. Total arrived at, 273.

Final spring check by Warden Updegraff, March 1, 1934. One hundred and forty-four flushed in 14 coveys and there were probably about 20 more on the basis of sign. Total arrived at, 164.

The exceedingly heavy drop in population during the winter was due to eviction of the coveys on practically the whole south half of the area through clearing of brush and burning. The northwest corner did not burn over and retained its quail population with little change throughout the winter, indicating very strongly that there was no influx of evicted birds into this part of the area, at least.

Area "B"—1179 acres. Sec's. 31, 5, 6, Wells Township, Appanoose county.

Pre-shooting check by Errington, Mr. and Mrs. Hamerstrom, Wardens J. C. Graham and C. H. Updegraff, November 18, 1933. Estimate of 875 birds, or one per 1.3 acres.

Experimental shooting November 22, 24, 27, 29, December 1, 4, 7, 9, 11, 13—Warden J. C. Graham in charge. One hundred and one birds bagged; 41 reported crippled or lost. Total known toll from shooting, 142.

Post-shooting and spring checks proved impossible to make with accuracy because of the denseness of cover in river bottomland and territories; however, the evidence pointed toward a strong surviving population.

Area "C"—1047 acres. Sec's. 7, 8, 17, 18, Roscoe township, Davis county.

No pre-shooting check.

Experimental shooting November 23, 25, 28, 30, December 2, 5—Warden Carl Hinkleman in charge. Ninety-eight birds bagged; 17 reported crippled or lost. Total known toll from shooting, 115.

Post-shooting check by Warden C. H. Updegraff, January 3, 1934. Two hundred and thirteen birds flushed, including one covey seemingly not assembled; the true total would probably be about 220.

Final spring check by Warden Updegraff, February 28. One hundred and ninety-nine flushed. A crisis brought on by heavy snowfall resulted in some starvation at this time; remains of 7 birds were found, of which 5 had obviously weakened to be caught by foxes.

Area "D"—965 acres. Sec's. 17, 18, 19, 20, Prairie township, Davis county.

Pre-shooting check by Errington and Wardens C. H. Updegraff and J. C. Graham, November 17, 1933. Ninety-eight birds flushed in 5 coveys; 14 calculated territories at 19.60 birds per covey gave 274 birds more. Total arrived at, 372, or one per 2.6 acres.

Experimental shooting November 25, 28, 30, December 2, 7, 9, 17—Warden W. E. Hicks in charge. Ninety-three birds bagged; 24 reported crippled or lost. Total known toll from shooting, 117.

Post-shooting check by Warden Updegraff, December 30. One hundred and thirteen flushed in 11 coveys; 5 calculated territories at 10.27 birds per covey gave 51 more. Total arrived at, 164.

Final spring check by Warden Updegraff, February 27. One hundred and eighty-six flushed in 13 coveys. Evidence of starvation in at least one covey.

Area "E"—1503 acres. Sec's. 13, 14, 7, 8, 17, 18, Salt Creek and Village townships, Davis and Van Buren counties.

Pre-shooting check by Errington and Wardens C. H. Updegraff and J. C. Graham, October 22, 1933. Thirty-seven flushed in 3 coveys; 6 territories at 12.33 gave 74 more, or a total of 111 for the uplands. On the

lowlands, 104 were flushed in 7 coveys; 6 territories at 14.85 gave 89 more, or a total of 193. Total for uplands and lowlands together, 304 birds, or one per 4.8 acres.

Experimental shooting, November 23, 25, 28, 30—Warden A. F. Meier in charge. Eighty-three birds bagged; 23 crippled or lost. Total known toll from shooting, 106.

Post-shooting check by Errington and Warden Updegraff, December 16 and 18. Fifty-eight flushed in 5 coveys; 3 territories at 11.60 birds per covey gave 35 more, or an upland total of 93.

Final spring check by Errington, Mr. and Mrs. Hamerstrom, Wardens C. H. Updegraff and J. C. Graham, February 25, 1934. Seventy-six flushed in 10 coveys; 2 territories at 7.6 birds per covey gave 15 more, or an upland total of 91. In the lowlands, 135 flushed in 10 coveys; 2 territories at 13.50 birds per covey gave 27 more, or a lowland total of 162. Total for area, 253.

Some evidence of poaching after the shooting season; evidence also of strong influx into the lowlands during the winter, apparently from across the river which bounds the area on one side.

Area "F"—968 acres. Sec's. 29, 30, 31, 32, Polk township, Jefferson county.

Pre-shooting check by Errington and Warden Updegraff, November 8, 1933. One hundred and sixteen flushed in 7 coveys; 22 calculated territories at 16.57 birds per covey gave 365 more. Total arrived at, 481, or one per 2 acres.

Experimental shooting November 22, 24, 27, 29, December 1, 4, 6, 8, 11, 13—Warden Otto Klinge in charge. One hundred and sixteen bagged; 38 reported crippled or lost. Total known toll from shooting, 154.

Post-shooting check by Warden Updegraff, December 26. One hundred and forty-seven flushed in 12 coveys; 4 territories at 12.25 birds per covey gave 49 more. Total, 196.

Final spring check by Warden Updegraff, February 20. One hundred and ninety-four flushed in 15 coveys; 2 territories at 12.93 birds per covey gave 26 more. Total, 220.

The southwestern part of the area, or that richest in birds, was burned off and a heavy population forced out. The evicted quail moved, to some extent, into the rest of the area, in one case massing into a covey as large as 40 birds. Evidence of predation, particularly by Cooper's hawks.

Area "G"—3600 acres. Sec's. 13, 14, 15, 22, 23, 24, 25, 26, 27, Liberty township, Jefferson county.

Pre-shooting check by Errington, early November. Fifty-four flushed in 4 coveys; 37 calculated or estimated territories at 13.50 birds per covey gave 500 more. Total arrived at, 554, or one per 6.5 acres.

Experimental shooting November 23, 25, 28—Warden C. H. Updegraff in charge. Sixty-seven birds bagged; 17 crippled or lost. Total known toll from shooting, 84.

Pre-shooting check on sample of about 1000 acres to be kept under observation, 162. Experimental shooting, calculated on a pro rata basis, took a toll of 25 on the observational sample.

Post-shooting check on the sample, by Errington, December 17. Forty-two flushed in 4 coveys; 5 territories at 10.50 birds per covey gave 53 more. Total, 95.

Subsequent checks by Dr. Carl Welty of Parsons College and student cooperators: December 28, 99 birds; February 19, 76; February 27, 115; March 10, 67; March 22, 84 flushed, possibility of about 20 more. Final total arrived at, 104.

The bulk of February-March fluctuations in population were apparently caused by the influx and egress of border coveys.

Area "H"—640 acres. Sec. 15, Van Buren township, Keokuk county.

Pre-shooting check by Errington and Wardens T. K. Johnston and C. H. Updegraff, November 6. Two hundred and sixty-five flushed in 17 coveys; 3 territories at 15.58 birds per covey gave 47 more. Total, 312, or one per 2 acres.

Experimental shooting November 22, 24, 27, 29, December 1, 4, 7, 9—Warden T. K. Johnston in charge. One hundred and twenty-eight birds bagged; 38 reported crippled or lost. Total known toll from shooting, 166.

Checks by Mr. and Mrs. Hamerstrom: January 20 and 21, either 88 or 93; February 9 and 10, 68; February 24 (with Errington), 75.

The food supply was very short, and at least 2 coveys near the edge were known to have moved out by the end of the winter.

Area "I"—1745 acres. Sec's. 23, 26, 27, Wayne township, Monroe county.

Pre-shooting check by Errington and Warden J. C. Graham, October 21 and 24. Seventy-four flushed in 5 coveys; 14 calculated territories at 14.80 birds per covey gave 207 more. Total arrived at, 281, or one per 6.2 acres.

Experimental shooting November 22, 24, 27, 29, December 1, 4, 6, 8, 11—Warden A. E. Miller in charge. One hundred and fifteen bagged; 34 crippled or lost. Total known toll from shooting, 149.

Post-shooting check by Warden C. H. Updegraff, December 23 and 25. Fifty-eight flushed in 7 coveys; 8 calculated territories at 8.28 birds per covey gave 66 more. Total arrived at, 124.

Final spring check by Updegraff, February 21. One hundred and five birds in 8 coveys.

Area was heavily pastured during the winter. Toward the last, practically the whole southeast half was quail vacant.

Area "J"—3180 acres. Sec's. 13, 23, 24, 25, 26, 34, 35, Urbana township, Monroe county.

No pre-shooting check.

Experimental shooting November 23, 25, 28, 30, December 9, 11, 13—Warden George Killinger in charge. Two hundred and three birds bagged; 14 reported crippled or lost. Total known toll from shooting, 217.

Post-shooting check by Warden Updegraff, December 27, 28 and 30. Five hundred and three birds flushed in 40 coveys; 6 territories at 12.57 birds per covey gave 75 more. Total, 578.

Final spring check by Updegraff, February 25, 26, 28, March 5, 7 and 8. Three hundred and seventy-four flushed in 24 coveys; 8 territories at 15.58 gave 125 more. Total arrived at, 499.

This was one of the few areas upon which the hunters really fed the birds all winter. Despite this, there was an apparent drop in number of resident birds, for no ascertained cause.

Area "K"—2508 acres. Sec's. 1, 2, 3, 34, 35, 36, 25, 26, 27, Lick Creek township, Van Buren county.

No pre-shooting check.

Experimental shooting November 23, 25, 28, 30, December 7, 9—Wardens C. H. Updegraff and George Killinger in charge. One hundred and thirty-four birds bagged; 18 reported crippled or lost. Total known toll from shooting, 152.

Only one check made subsequently, by Errington and Updegraff on a representative sample of about one square mile, January 11. Sixty-three birds in 6 coveys; 3 territories at 10.50 birds per covey gave 32 more. Total, 95.

Area "L"—1931 acres. Sec's. 26, 27, 34, 35, Highland township, Wapello county.

Pre-shooting check by Errington, Wardens Updegraff and Graham, October 19 and 20. Forty-five flushed in 3 coveys; 18 territories at 15 birds per covey gave 270 more. Total arrived at, 315.

Experimental shooting November 23, 25, 28, 30, December 2, 5, 7, 9, 11—Warden George Gibson in charge. Thirty-six birds bagged; 7 reported crippled or lost. Total known toll from shooting, 43.

Post-shooting check by Updegraff, December 22 and 24. One hundred and sixty-seven flushed in 12 coveys; 7 territories at 13.91 birds per covey gave 97 more. Total, 264.

Final spring check by Updegraff, March 10 and 11. One hundred and eighty-three in 13 coveys; 4 territories at 14.07 birds per covey gave 56 more. Total, 239.

Much of the north and central part of the area was burned over and many birds were consequently evicted from established territories.

Area "M"—3406 acres. Sec's. 32, 33, 34, 9, 10, 3, 4, 5, Center and Richland townships, Wapello county.

No pre-shooting check.

Experimental shooting November 22, 24, 27, 29, December 1, 4, 7, 9—Warden M. D. Lewis in charge. Seventy bagged; 21 reported crippled or lost. Total known toll from shooting, 91.

A final spring check was made by Updegraff on a sample area of 400 acres of bottomland type. He flushed 101 in 8 coveys (March 2, 3 and 15); 2 possible territories at 12.62 birds per covey gave 25 more. Total surviving on the sample, 126, or one per 3.2 acres.

Area "N"—740 acres. Sec's. 33, 34, Jefferson and Grand River townshipe, Wayne county.

No pre-shooting check.

Experimental shooting November 22, 24, 27, 29, December 7, 9—Warden H. A. Holmgren in charge. Sixty-two bagged; 70 reported crippled or lost. Total known toll from shooting, 132.

Post-shooting check by Mr. and Mrs. Hamerstrom on about half the area, January 27 and 28. Fifty-seven birds flushed.

Final check by Mr. and Mrs. Hamerstrom, February 3 and 4. Good count of 116 flushed.

UNSHOT AREAS

Area "O"—Sample 300 acres of a 1400 acre area, Des Moines City Waterworks Supply Grounds Wild Life Refuge. All counts by Errington.

December 8, 71 were flushed in 4 coveys; 2 territories at 17.75 birds per covey gave 36 more or a bird per 2.8 acres. Total, 107.

January 12 and 16, 88 birds in 7 coveys; 1 territory at 13 birds gave a total of 101.

February 27 check; 99 in 7 coveys.

This was an area in which winter feeding was handled more efficiently than in any other area under observation. The population was well established, with the exception of a small covey of 7 which seemingly wandered until destroyed. Evidences of only 2 dead birds were found.

Area "P"—An area of about 500 acres north of the State College at Ames.

December 13, by Errington, flush count of 42 in 3 coveys or a bird per 12 acres.

Early January count of 43 by Mr. and Mrs. Hamerstrom.

February 27, a count of approximately 38 by Hamerstrom; evidence of one dead bird.

Area "Q"—Area of about 1500 acres, 2 miles east of Bloomfield.

December 31, Warden Updegraff flushed 164, in 13 coveys, and recorded 10 territories which at 12.61 birds per covey gave 126 more. Total arrived at, 290 or about a bird per 5 acres.

March 3 and 4, Mr. and Mrs. Hamerstrom obtained a flush count of 137 plus another covey of about 20 and another of about 16. Total, 173. Seven fresh kills of starving birds and 3 older kills found on this visit.

This area had been seriously burned and cleaned up.

Area "R"—Sample of about 400 acres of Fort Des Moines state game management (U. S. Army property).

This area had been under partial cultivation, but has been allowed to revert since the summer of 1932. The reversion was followed by a tremendous increase in smartweeds, pigeon grass, and other vegetation productive of quail food; the subsequent ecological succession has been unfavorable to quail food plants, however, thereby reducing materially the carrying capacity of the range. The feeding stations and food patches present were insufficient to offset more than partially the attractiveness of the cultivated fields of the area.

The December 11 check by Errington totalled about 60 birds, or a bird per 6.6 acres on the basis of birds flushed and evidently occupied territories, as compared with a New Year's population of about an even 100 for the season of 1932-33.

On January 8, Errington and Warden George Killinger made an unsatisfactory check which indicated that a large border covey had moved out and that the remaining population certainly did not exceed 40 birds.

Killinger's final check, February 27, gave 24.

Only evidence of mortality recorded was one bird killed by a Cooper's hawk.

Area "S"—Area of 800 acres about ten miles south of Ottumwa.

First check by Errington and Mr. and Mrs. Hamerstrom, November 19 and 21. Flush of 90 in 6 coveys in north portion; 1 additional territory. Flush of 56 in 5 coveys in south portion; 3 territories in addition. For the entire area 146 birds were flushed in 11 coveys; 4 territories at 13.27 gave 53 more. Total of 199 or a bird per 4 acres.

Final check by Errington February 19 and 20. Flush of 83 in 7 coveys in north portion; flush count of 54 in south portion, with 1 extra territory.

For the area as a whole, 137 flushed in 12 coveys and 1 covey territory at 11 birds gave a total of 148.

Area "T"—3200 acres. Area east of Prairie du Sac, Wisconsin, which has been under observation since 1929.

The census figures were obtained by Albert J. Gastrow of Prairie du Sac, a man well qualified by experience for this work and who spent an average of three days a week in the field from October, 1933, to April, 1934.

Since this is the most important individual area which we have studied, and since the work was carried on with exceptional accuracy, it may be advantageous to present the data in condensed detail. The groups used as headings refer to populations of quail usually isolated to some degree, or segregated for convenience in handling the data.

Group I. November 13, 19+14 birds; January 3, 18+14; February 14, 16+13; March 22, 13+12. Loss of 8 of 33 in 128 days.

Group II. October 23, 11+14 birds; November 10, 12+12; February 14, 12+11; February 27, 8+11. Loss of 6 of 25 in 127 days, one of which was presumably a Cooper's hawk kill. Food shortage seemed to bring on something of a late winter crisis.

Group III. October 31, 24; November 13, 23; December 12, 22; January 21, 21; February 12, 16; February 18, 12; March 3, 9; March 22, gone. Loss of 15 of 24 in 123 days; possibly nearly annihilated in the next 19 days. This group plainly occupied a lethal territory (for previous history see Errington, 1933a, group XXXII; 1933d, group V).

Group IV. October 21, 11+17; November 14, 7+18; December 2, 10+14; December 14, 23; January 16, 11+17 (influx from somewhere, possibly from outside area); February 15, 9+18; March 27, 7+15. Loss of 11 of 28; gain of 5; net loss of 6 in 156 days.

Group V. November 3, 14; November 28, 13; influx of a covey of 12 (possibly from VI) January 5; February 6, 11+13; February 22, 10+12; March 15, 9+12. Loss of 5, gain of 12; net gain of 7 in 132 days.

Group VI. December 8, 18+22; January 9, 25 (12 of these may have joined V); February 10, 24; February 20, 21; March 19, 20. Loss of 20 of 40 in 100 days.

Group VII. November 21, 24+18+17; December 10, 23+19+16; February 10, 21+16+12; March 31, 17+14) third covey probably left). Loss of 28 of 59 in 130 days.

Group VIII. November 23, 5; January 18, 3 or 4; thereafter gone, probably from area.

Group IX. November 7, 18 (this covey had numbered 27 from October 22 to November 2, but the others—mostly young—apparently left; at this season they could have gone nearly anywhere without detection); March 29, 18. No loss, but minor fluctuations in 141 days.

Group X. November 17, 15; February 2, 14; March 31, 13. Loss of 2 of 15 in 133 days.

Group XI. November 18, 8; January 5, 9 or 10; January 13, 15; February 12, 14; March 5, 11; March 31, 11. Loss of 4, gain of 7; net gain of 3 in 132 days.

Group XII. November 18, 15+11+12; November 25, 15+20; December 23, 13+14; January 11, 10+16; February 8, 20; February 27, 17;

March 22, 22. Suspected egress to XI in late November, influx from XIII in early March. Loss of 21, gain of 5; net loss of 16 in 123 days.

Group XIII.   November 21, 16; December 16, 20; January 9, 17; February 10, 21; March 3, 16; March 31, 16. Loss of 8, gain of 8, no net change in 129 days.

Group XIV.   October 26, 15; December 26, 13; December 30, 15; January 11, 17; February 8, 17; March 5, 15; March 17, 14. Loss of 5, gain of 4; net loss of 1 in 141 days.

Group XV.   December 6, 12; January 9, 12; disappeared by February 10, apparently to be dissipated largely in groups XIII and XIV. Loss of entire covey in 66 days.

Group XVI.   November 2, 16+18; November 25, 15+17; February 4, 14+10; March 26, 10+13; March 29, 11+12. Loss of 11 of 34 in 146 days.

Group XVII.   October 14, 17+18+14; February 16, 14+11+10; by about March 25, 15+8+10. Loss of 16 of 49 in about 161 days.

The total loss for area "T" was 145 of 433, of a bird per 7.4 acre population, or 35.8 per cent for an average of 132 days, leaving a surviving population of 288 birds in an area which showed, during the peak population winters of 1931-32 and 1932-33, a carrying capacity of about 330, or a bird per 9.7 acres.

The drop in survival may be reasonably attributed to the eviction of the equivalent of two coveys by the roadside debrushing activities of the C. W. A. All roadside brush and even some along private driveways was cut away, some of which was highly important quail cover.

COMPARISON OF WINTER MORTALITY RATES ON SHOT AND UNSHOT AREAS

Not all of the shot areas are eligible for comparison with those unshot because of lack of data, and emergencies on others brought about addi-

Winter population changes on shot areas

Area	Post-shooting Check	Final Check	Loss (+gain)	Salient remarks
A	273	164	109	One-half area burned
C	220	199	21	Detected mortality: starvation.
D	164	186	+22	Some starvation.
E uplands	93	91	2	Strong influx on bottomlands, but no post-shooting check.
F	196	220	+26	Heavy burning, considerable predation.
G	95	104	+9	Considerable egress and influx at border.
H	93	75	18	Food shortage. Two border coveys moved.
I	124	105	19	Heavy pasturing. Half of area quail-vacant.
J	578	499	79	
L	264	239	25	Much burning.

tional complications. Areas "B", "E" (lowlands), "K", "M" and "N" were eliminated from consideration because of inadequate censuses. The data from the ten remaining shot areas are summarized in the foregoing table.

The total post-shooting winter decrease recorded on the shot areas amounted to 216 of 2100 birds or 10.3 per cent for an average of 62 days.

Before comparing the data of the shot and unshot areas, a critical inquiry as to the causes of their heterogeneity is in order.

The drastic burning and debrushing, general food shortage and excessive pasturing which characterized the winter brought in variables, but variables operative both on the experimental areas and on the adjacent lands. The heavy burning of parts of areas "A' and "L", and the excessive pasturing of part of area "I" resulted in known eviction of whole coveys from their established territories. That birds moved into individual areas is shown by the data from areas "D" and "F".

The several censuses from area "G" reflect changes in population dependent upon whether border coveys were in or out of the area at the time of the check. It must be pointed out, however, that area "G" represents an exceptional case, in that the boundary of the sample under observation ran through a river bottom with an unusually high concentration of quail; hence, that portion actually on the area did not have a definitely isolated population.

In general, we feel, however, that the numbers of individuals under observation tend to compensate for errors because of the influx and egress of shifting populations and the presence or absence, at the time of the census, of border coveys.

Winter population changes on unshot areas

Area	Early Winter check	Final check	Loss	Salient remarks
O	107	99	8	Population excellently situated except for 1 covey.
P	43	38	5	
Q	290	173	117	Some starvation mortality.
R	60	24(?)	36	Loss due to egress rather than mortality.
S	199	148	51	
T	433	288	145	Area plainly over-populated.

The winter's decrease on the six unshot areas totalled 326 of 1132, or 31.98 per cent, over an average of 99 days. Omitting from consideration the out-of-state area "T" and the perhaps questionable figures from area "R", the loss of 181 of 639 birds, or 28.33 per cent, for an average of 68 days is nearly three times the 10.3 per cent average for the shot areas.

Predator populations were not noted to differ significantly on the southern Iowa quail areas, and, except for fur trapping and fox hunting

and sporadic hawk and owl shooting by hunters and farmers, predators were not greatly molested.

Area "T" had a predator population somewhat lighter than usual, but quail losses were the highest yet recorded, for the reason that there were more quail in excess of carrying capacity than there had been before. Exceptionally concerted hawk shooting was known to have been done on area "H", and we have well founded suspicion that the horned owls were cleaned out of area "R"; in neither case could any improvement of the situation be said to have become apparent. Conversely, on Area "O", a wild life refuge where native predators are encouraged along with other species of interest, but where the fundamentals of quail management may find actually practiced, the quail survival was excellent, save for one small wandering covey which appeared to be crowded out.

Studies of the food habits of the great horned owl (*Bubo virginianus virginianus* Gmelin), which we consider the bob-white's most efficient resident winter enemy, on the shot areas "A", "G", "H", "K", "L" and "N" disclosed negligible pressure from this source, thus indicating a decided security in the case of the quail populations surviving the shooting. Horned owl pressure, according to data in preparation for another paper, seems quite satisfactorily to reflect bob-white population vulnerability. Horned owl pellets (Errington 1930; 1932) from southern Iowa and Wisconsin observational areas having quail populations within carrying capacity, ordinarily contained quail remains in 2 per cent or less, compared to a representation as high as 15 per cent in pellets from overpopulated areas.

The data point toward an increased security for those quail which survived the shooting. The populations which were reduced by artificial means to carrying capacity, or below, suffered but little loss through predation during the winter. On the other hand, the surplus birds from the unshot areas were apparently removed by predators.

This season's data, therefore, tend to confirm those already published (Errington 1934) to the effect that the kinds and number of predatory species make little difference in winter quail survival, compared to the carrying capacity of the land. If the quail population is within the carrying capacity, it will be relatively secure against predators; if there are more quail than the environment can accommodate, predators will take them, if the surplus is not removed otherwise.

BIOLOGICAL AND MANAGEMENT ASPECTS OF SHOOTING

Vulnerability of the Iowa 1933 fall quail population began to be manifest about the first week in November. Prior to that time, field work uncovered scant evidence of losses, but mortality seemed to be greatly accelerated by the drying up of herbaceous vegetation and the dropping of leaves from deciduous brush.

It seems reasonable that the carrying capacity of early fall environment, when plant growth is green and fairly well distributed, is normally high for grown healthy birds, and a large part of the population may find habitable territories that would not be habitable for them later. The "fall shuffle" (Leopold 1931; 1933) may be interpreted as the reorientation of populations in response to autumnal changes in the environment. The mechanism of territorial adjustment is not clear, but instances have been

recorded (see Errington 1933c) of fall strife between coveys, conceivably over territory; increasing evidence, moreover, indicates that winter coveys have a tendency to avoid coverts already well populated, even when confronted by considerable or dire extremity.

When the carrying cacapity of an environment is lowered much below the resident population naturally (as in the fall) or artificially (as by wholesale burning, debrushing, or anything else which drastically affects solid blocks of land), the surplus or evicted coveys station themselves in inferior environment or move away. In either case, the end result to be expected is disproportionately rising mortality. Wandering coveys particularly suffer the pressure of the environment, and although complete annihilation is usually averted by a joining of the remnants to other coveys, the usual consequence is merely to over-populate by that many birds the stronger environments. A critical examination of the data from area "T" may give a better idea of how this happens.

The removal of a substantial portion of the fall population by shooting evidently relieved the winter environmental pressure upon the shot population considered as a whole, in spite of complications brought on by burning and food shortage. The lowered rate of loss is not to be mistaken for an equivalent rate upon a correspondingly lower quail density: the decided lowering of percentage of loss signifies increased security.

Nevertheless, the impression is not to be gained that shooting is a biological necessity. It provides only a means by which man may utilize or harvest an annual surplus which would otherwise be lost. Man need not consider himself obliged to shoot the surplus—if he does not, it will be removed naturally, as it always has been.

The intelligent harvesting of a quail crop is in itself not a simple matter. In the first place, we cannot say with too much certainty how many birds may be removed, still permitting complete population recovery during the breeding season, although our limited data indicate that the re-

Table showing unexplained population differences following shooting

Area	Pre-shooting check	Known toll	Post-shooting check	Unexplained differences	Reliability of difference data
A	425	124	273	28	Fair.
D	372	117	164	91	Fair
E uplands	111	38 pro rata	93	+20	Good except for pro rata
F	481	154	196	131	Fair.
G sample	162	25 pro rata	95	42	Pro rata data poor
H	312	166	93	53	Pre-shooting data excellent.
I	281	149	124	8	Fair.
L	315	43	264	8	Questionable.
Total	2459	816	1302	341	

covery rate varies inversely with population density in relation to environmental carrying capacity (Errington, 1934).

It is plain enough that the net toll of shooting upon the species materially exceeds the number brought to bag. According to the official report on the experimental shooting (Schuenke 1933), 1396 birds were bagged and 386 reported crippled or otherwise lost. The known toll, according to these figures, is 1782. (For a fuller account of crippling losses, see also Errington and Bennett 1933.)

That the toll from shooting may in actuality have been much heavier than the 1782 is borne out by the pre- and post-shooting population figures from the experimental areas.

At the completion of the first post-shooting census there was an unexplained loss of 361 birds from seven areas and a gain of 20 in the case of one area, or a net difference of 341 birds less than the figures from the original census, minus the known toll, would lead one to expect. It may be difficult or even impossible to explain these differences fully, but a discussion of the salient facts may be of advantage.

The fact that the pre-shooting censuses were not of uniformly high reliability permits the possibility that error may enter into the explanation of these differences. However, the relation between unexplained difference and original population in the case of area "H", with a pre-shooting census known to be accurate, compares closely to the same relation applied to the data as a whole, 16.99 per cent and 13.88 per cent, respectively. This close relationship would seem to indicate that the explanation does not lie wholly in errors of censusing, but that we must look farther.

It is known that the pressure of heavy shooting can cause quail to move from their established territories (Stoddard, 1931). That a portion of the difference was due to this cause may be accepted as a fact. It is highly probable that a portion—and perhaps a very large portion—of these differences may be attributed to wounded birds which were not recognized as such, but which later died as a result of their wounds, or, in their weakened condition, fell easy prey to predators. These possibilities seem the most reasonable explanations, and, in anticipation of them, the first post-shooting checks were made after the lapse of sufficient time to permit populations to reach equilibrium.

SUMMARY

1. Quail populations surviving the shooting lost 10.3 per cent of their numbers collectively during the period of observation.

2. Quail populations on the four reliable Iowa unshot areas, comparable in location and period of observation to those shot, lost 28.3 per cent.

3. Predator populations were not noted to differ significantly on the Iowa experimental areas, shot or unshot.

4. Mortality seemed to be greatly accelerated in early November, coincident with the reduction of carrying capacity by the drying up of herbaceous vegetation and the falling of leaves from deciduous brush.

5. The first post-shooting censuses showed fewer birds than the figure obtained by subtracting the known toll from the pre-shooting population.

The data appear to confirm the population vulnerability thesis (Er-

rington, 1934) in that they indicate heavy predation upon the exposed sur-
pluses of the unshot areas, while those areas from which the surplus was
artificially removed showed a lower loss rate.

While it is not our part either to advocate or to oppose quail shooting
for sport, the data show that reasonable shooting is biologically possible,
and, if it is to be done, would be most advantageous in early fall, in order
that the kill might comprise birds from the doomed seasonal surplus.
Later in the winter, after the surplus had long been exposed to natural
mortality, the same kill might reduce the population too far below the
carrying capacity of the environment and reduce not only surplus but
seed stock as well. In the matter of setting bag limits, however, it should
not be overlooked that the number brought to bag does not represent the
true toll upon the species.

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HISTOLOGICAL STUDIES OF THE DEVELOPMENT OF THE ROOT AND CROWN OF ALFALFA[1]

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Several investigators, notably de Bary (1), van Tieghem (7, 8), Compton (2), Jones (4), and Winter (9), have described the vascular anatomy of the primary root and hypocotyl of alfalfa and Jones has followed the anatomical development of the crown. The descriptions are not entirely uniform and a number of structural features of the root and hypocotyl such as the origin of the phellogen, sloughing of the primary cortex, development of the secondary cortex, development and thickness of the cork, and the nature of the libriform fibers have received little if any attention at all.

The object of the investigation, herein reported, was to ascertain the anatomical development of *Medicago sativa* from embryo to maturity under conditions where the plants were not mowed or pastured. This information, it is hoped, will be of value in determining the disturbances in the structure of the plant caused by mowing and pasturing, and also in better understanding the relation of disease and winter killing to the abnormalities in anatomy resulting from methods of management.

LITERATURE

The xylem of the young primary root of *Medicago sativa* is prevailingly triarch, according to van Tieghem (7), de Bary (1), and Winter (9). Exceptions in which tetrarchy was observed have been reported by both van Tieghem (8) and Winter (9). According to Winter (9) the upper part of the primary root commonly becomes tetrarch with age, thus approaching the condition of the hypocotyl immediately above where tetrarchy prevails. In Compton's (2) investigations of the seedling anatomy of legumes, including *Medicago lupulina* L., *M. falcata* L., *M. turbinata* Willd., *M. trunculata* Gaertn., *M. tribuloides* Desr., he found a triarch primary root in the first three.

In regard to the cotyledonary traces Winter claims that they are continuations of the vascular elements of the primary root and that each trace is triad in constitution, consisting of a polar xylem (xylem strands in the vertical plane of the cotyledons) and a part of each lateral xylem group. She found that the primary xylem disappeared from the traces as they approached the lamina of the cotyledons where there was a fusion of flanking xylem strands. According to her observations the primary vascular system of the root, hypocotyl, and cotyledons constitute a system separate from that of the plumule which develops later and is superposed upon the vascular system of the hypocotyl.

Concerning the hypocotyl Jones (4) states that the xylem groups are separated by masses of parenchyma which at the upper end of the hypocotyl sometimes form a pith-like center. He found, however, that this pith-like center was not continuous wtih the true pith of the plumule but was separated from it at the node of the unifoliate leaf by the closing together of the vascular elements and the formation of a woody plate.

In *Medicago falcata*, Gerard (3) observed that of the three vascular strands of the root two lead into the cotyledons and the third into the first leaf.

The contraction of the roots and hypocotyl of *Medicago sativa* has been investigated by Rimbach (6) and Jones (4). Rimbach recorded 50 per cent shortening of these structures in the first five months of the plant's development. Rimbach observed that the contraction in length extended to the secondary and even the tertiary roots. According to Jones the contraction in length of the hypocotyl and probably of the upper part of the root is due to the radial expansion of the parenchyma tissue in the center of the stele and of that interspersed among the vascular elements. As a result of the lateral expansion of the parenchyma, the fibers and conductive strands in the stele are forced into sinuous courses and thus shortened lengthwise. In accommodating themselves to the lengthwise shortening of the vascular strands the entire hypocotyl and root are contracted.

In his investigations of the anatomy of the crown, Jones (4) observed that the base of the stem had characteristics in its primary elements of a transition structure between root and stem. At the base of the stem the endodermis was well defined but disappeared at higher levels. He noted that the xylem tissues of the bundle as well as the vascular rays became heavily lignified and formed a cylinder of strengthening tissue. He found that the cambium in the base of the axillary stems was very active, and that cells added to the central cylinder during the early activity of the cambium became heavily lignified, whereas, those added later took on modifications that made them indistinguishable from the cells formed in the root. The interfascicular cambium of the crown branches formed rays similar to those of the root and these rays also stored starch. The crown branches were found to closely resemble roots in structure, but they were capable of producing new buds.

Jones (4) noted that in the stem bases of the crown the phellogen originated in the endodermis. He observed also that following the formation of the phellogen the epidermis of the stem bases died, separated from the cortex, and became the brown membranous covering often noticeable on the bases of the young stems early in the spring.

Jones (4) found that the annual growth of the tap root, especially in its upper part, was recognizable as growth rings by the contrast in size of the fall and spring vessels.

<div align="center">EXPERIMENTAL</div>

<div align="center">MATERIALS AND METHODS</div>

Preliminary examinations of young plants collected from both the field and the greenhouse showed no noticeable anatomical differences. Consequently, material for the study of the early stages was collected

indiscriminately from either place. For the study of the older stages the material was confined to plants grown in the field. The roots and crowns for the anatomical studies were collected from plants of approximately the following ages, counting from date of planting: 5 days, 10 days, 15 days, 20 days, 30 days, 40 days, 50 days, and 60 days, three months, four months, five months, six months, one year, two years, three years, and four years.

For some of the histological work, temporary preparations were satisfactory, while the study of the finer structures required paraffin and celloidin sections. Material older than 50 days was so woody that it was necessary to imbed it in celloidin. Material 20- to 50-days-old was satisfactorily sectioned in paraffin when cedar oil was used in the infiltration and the paraffin blocks were soaked in warm water.

Bouin's killing fluid was most satisfactory for all ages. However, one per cent of chrom-acetic gave good results on one-year-old plants and Licent's fluid gave best results for five-day-old material when the pieces were about two millimeters in length. Penetration was poor in longer pieces.

The staining reaction varied greatly for different ages of material, consequently several combinations of stains were used. For staining the early stages, aqueous safranin and fast green in 95 per cent alcohol gave best results. For older material, three different combinations of stains were used: aqueous safranin and Mayer's haemalum or safranin and gentian violet, both in clove oil, and safranin with fast green in 95 per cent alcohol. In most cases the safranin and gentian violet in clove oil gave the clearest differentiation of tissues.

The features of the libriform fibers which are best observed in cross-sections were most satisfactorily studied in sections on permanently prepared slides. The length of the fibers was determined from measurements of isolated fibers in macerated material. The maceration was accomplished by a preliminary treatment in warm 10 per cent sodium hydroxide for 30 minutes, followed by thorough rinsing and a treatment with potassium chlorate and nitric acid.

All of the figures were drawn with the aid of the micro-projector.

ANATOMY OF PRIMARY ROOT

The rate at which the seedlings of alfalfa developed, especially during the early period of growth, varied considerably with the moisture, temperature, and the length of day. The influence of these factors was especially noticeable in plants grown in the greenhouse at different times of the year. Differences in rate of growth were accompanied by corresponding differences in age at which both primary and secondary structural features appeared. The description of the anatomical status of the plants at the given ages were considered quite generally applicable to plants grown under average conditions of moisture, temperature, and light.

The alfalfa embryo, while still in the seed, possesses two meristematic tips on the apex of the hypocotyl (fig. 1, B). The one more apical and conical is the shoot primordium. The other one, which is the primordium of the first or unifoliate leaf, is at one side of but close to the shoot primordium. The primordium of the first leaf is connected by procambial initials with those of the hypocotyl and radicle. It also precedes the
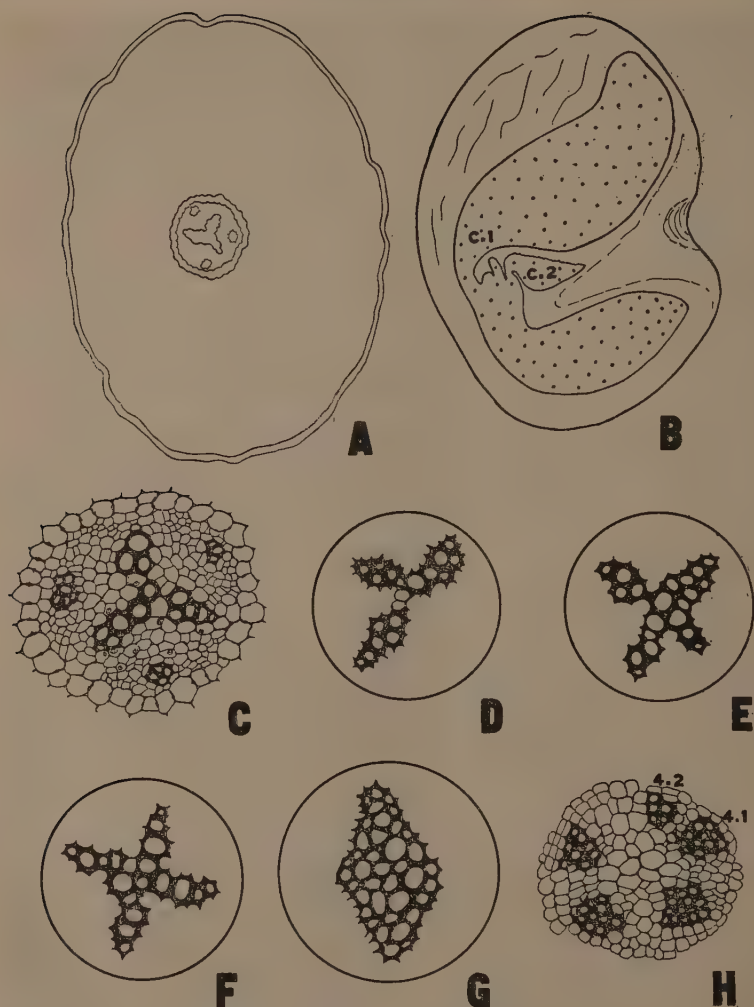
Fig. 1. A. Drawing to show the size of the stele in relation to the entire primary root.
  B. Longitudinal section through an alfalfa seed. C1 and C2 are cotyledons. The two meristematic tips are shown between them.
  C. Detailed drawing showing the primary structure of the stele of the tap-root.
  D. to H. Stages in the vascular transition of some plants from the root up to the base of the cotyledons.
  D. Two of the vascular bundles which have become widely separated.
  E. A new vascular bundle being formed.
  F. Fourth bundle formed.
  G. Lignified metaxylem completely filling the arcs between the rays.
  H. Section through the hypocotyl where the xylem has become divided to form the individual bundles. One bundle has further divided to form 4.2 the bundle to the first leaf.

shoot meristem in further development, which delays the formation of the shoot until after the first leaf is well formed. In five-day-old seedlings grown under average conditions of light, moisture and temperature most of the primary structures characteristic of alfalfa roots were present. Hence, in the discussion of older stages the attention can be confined largely to the secondary structures.

The primary vascular system of the root in seedlings five days of age was quite generally a triarch protostele of the exarch type (fig. 1, C). This was previously reported by Winter (9). The plants found with a tetrarch arrangement in the primary root were relatively few. The secondary roots so far as observed were diarch. Except in the transitional region in the upper part of the roots the three xylem arcs in the primary triarch roots were symmetrically placed with angles of about 120° between them.

The differentiation of vascular elements was observed to begin in the cells near the pericycle and to proceed centripetally until the three xylem arcs united, thus forming a pithless stele. The vascular elements were recognizable less than a millimeter behind the growing tip. The protoxylem elements consisted of reticulate and pitted tracheae.

In the protophloem of five-day-old plants it was difficult to recognize the tissues typical of well-formed phloem. Neither sieve tubes nor companion cells could be distinguished. There were, however, three groups of phloem fibers located between the rays of xylem and in contact with the pericycle. These fibers were differentiated early, appearing soon after the protoxylem. They varied considerably in thickness of walls and in reaction to stains in different seedlings.

The stele was surrounded by a definite endodermis composed of thin-walled cells. Near the tip of the root the cells of the endodermis were more or less circular in transverse section, while in the older regions of the root they were decidedly oval. Small casparian strips were clearly recognizable only in transverse sections in which the tissues were well developed. The pericycle, as far as observed, consisted of one layer of parenchyma cells, and was in direct contact with the fibers of the phloem strands, but separated from the protoxylem by one or more layers of parenchyma.

In the roots of seedlings five days old, the stele occupied a relatively small proportion of the root's cross-sectional area, its diameter being only about one-seventh of that of the entire root (fig. 1, A). At this stage the primary root consisted mostly of primary cortex. In the outer region of the primary cortex the cells were relatively large and loosely joined, whereas in the region of the endodermis the cells were much smaller and more compactly joined.

VASCULAR TRANSITION FROM ROOT TO CROWN

In the upper part of the root where the transition from root to hypocotyl occurred, the xylem arcs were so related that two of them fell in the same straight line while the third met them at an angle of approximately 90° (fig. 1, D). Near the enlarged portion or collet of the root, which, as a rule, was immediately above the upper limit of the root hair region, a beginning of a fourth arc near the center of the large angle was present in seedlings five days of age (fig. 1, E). In this region, the casparian strips
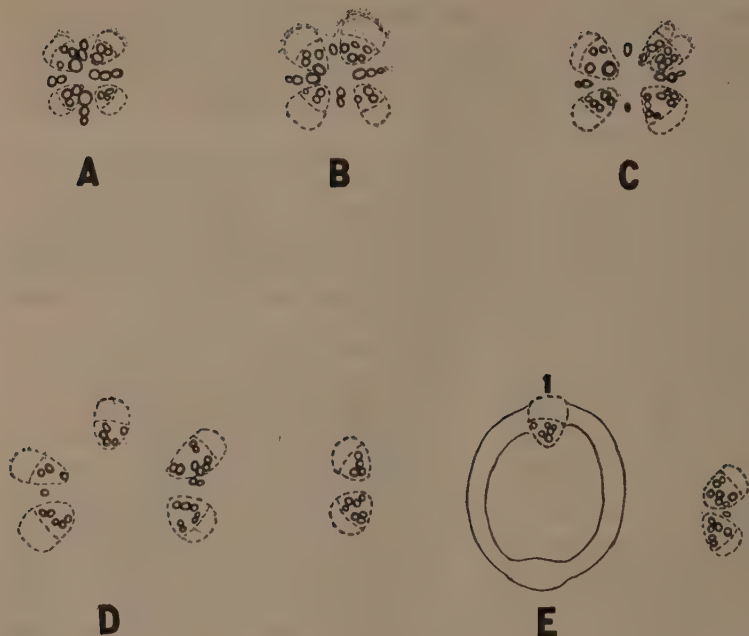
Fig. 2. Another transition scheme, a series from five-day-old plants in which the entire xylem area did not become lignified. The dotted lines enclose the meta-xylem masses and between them are the primary tracheae.

A and B. Cross section of the upper part of the primary root or lower part of the hypocotyl showing lignified metaxylem cells between the primary xylem arcs.

C. Cross section of hypocotyl showing a radial row of tracheae (r) in one of the four masses of metaxylem.

D. Cross section of hypocotyl showing groups of xylem vessels widely separated with some primary tracheae between them.

E. Section through upper hypocotyl showing cotyledonary traces and the trace (1) leading to the first leaf.

of the endodermis were absent and cambial initials were recognizable The cambium was differentiated basipetally and at five days of age not much secondary growth was present except in the older tissues of the hypocotyl near the base of the plumule.

A few millimeters above the place of origin of the fourth arc of xylem, some differentiation of metaxylem had occurred at five days of age, but the amount of lignification varied considerably in different plants. In some plants the regions between the four xylem arcs were entirely filled

with lignified cells, thus forming a diamond-shaped mass of xylem (fig. 1, G), while in other plants only a few lignified metaxylem cells scattered among the parenchymatous cells between the xylem arcs were present (fig. 2, A). Still farther, five or more millimeters, above the origin of the fourth xylem arc, a radial row of tracheae was differentiated in one of the four masses of metaxylem (fig. 2, C). Serial sections farther up in the hypocotyl showed that this radial row of tracheae separated from the remaining mass of metaxylem and passed alone into the first leaf. When the xylem was a solid cylinder in the lower part of the hypocotyl, it separated into two masses of xylem higher up in the hypocotyl, and the region between the two masses of xylem became filled with pith-like parenchyma. A little farther up in the hypocotyl these two xylem masses became further divided into four groups of metaxylem, while one or two tracheae of each of the primary arcs could be seen imbedded in the parenchymatous tissues separating the metaxylem groups (fig. 2, D).

The two primary arcs which were oriented in directly opposite directions were in the vertical plane of the cotyledons as noted by previous investigators, who refer to them as polar xylems (fig. 1, D). The other two bundles of the tetrarch group in the hypocotyl, known as inter-cotyledonary bundles, extended at right angles to the line of attachment of the coteyledons (figs. 1, E, F).

The polar primary xylems, after extending a short distance into the bases of the cotyledons, were each displaced by a bundle of metaxylem. The two metaxylem bundles remained separate until well up in the lamina of the cotyledon, where they fused and became a part of the midrib (Pl. I, fig. 5, and Pl. II, fig. 4). The inter-cotyledonary bundles of the primary root terminated in the hypocotyl before the cotyledonary node was reached. The radial row of tracheae previously mentioned as separating from the mass of xylem in which it was initiated and extending into the petiole of the first leaf, continued in the vertical plane of the inter-cotyledonary primary bundles.

The secondary tissues which had formed in the upper part of the hypocotyl above the place where the cotyledonary traces separated, formed the new vascular tissues of the plumule. As the vascular strand of the first leaf moved toward the periphery of the hypocotyl, more tissues were formed about its margin, resulting in a complete cylinder of vascular tissues having pith in the center (fig. 2, E, and fig. 3, A). At a higher level there were two or three vascular bundles in the process of differentiation on opposite sides of the vascular strand of the petiole. These were the primordia of the vascular system of the stipules of the first leaf (fig. 3, B). The vascular bundle of the second leaf was formed directly opposite that of the first leaf and was easily distinguished from the stipular bundles by its larger size.

CHANGES ACCOMPANYING SECONDARY GROWTH

SECONDARY XYLEM STRUCTURES

The first appearance of secondary xylem in the regions of the root where the primary xylem was typically triarch occurred about the fifteenth day after planting. The secondary xylem was formed first in the angles between the primary xylem lobes. In the upper part of the root
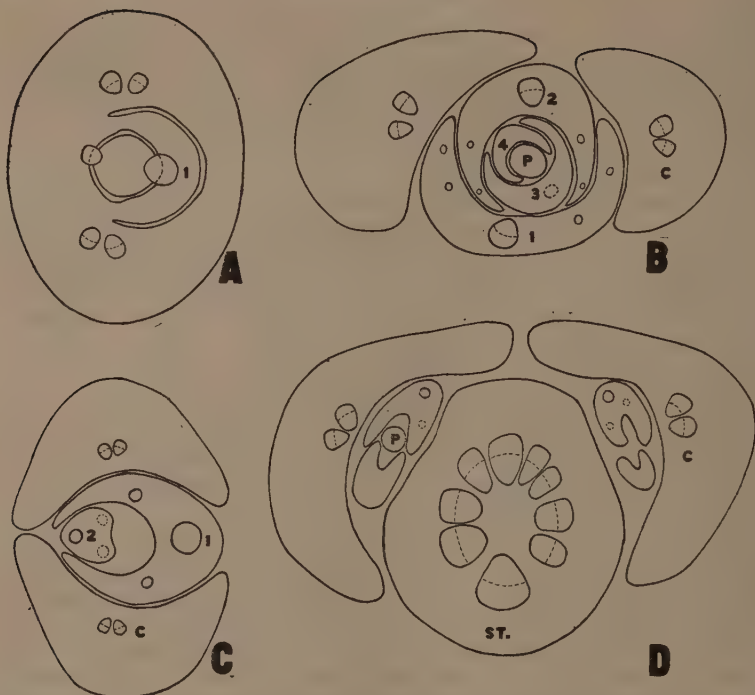
Fig. 3. A. Vascular tissues can be seen opposite (1) for the petiole of the second leaf.
         B. Cotyledon bases C, a separate structure. First leaf petiole 1, has stipular
             tissue attached with the vascular bundles enclosed. 2, petiole of the sec-
             ond leaf.
         C. Section from plant 15 days old. Numbers 1 and 2 are petioles of the leaves
             with the adherent stipules extending to each side of them. P, the plumule
             of the short meristem. C, cotyledons.
         D. Section through the crown of a 21-day-old plant. C, a cotyledon base
             with a shoot developing in the axil. St, stem structure.

where two of the xylem lobes were placed at approximately 180° to each
other, formation of the secondary xylem was at first most rapid in the
large angle until the stele became cylindrical, after which growth was
more uniform about its circumference.

Libriform fibers first appeared in the xylem approximately 20 days
after planting. They varied in number in different plants and were formed
in groups in close proximity to the tracheae and at approximately the
same time as the tracheae. The formation of the libriform fibers was first
recognizable a few layers of cells beneath the cambium. It was also
noticeable both in young and old plants that there was an association of
libriform fibers and parenchyma-like cells which contained crystals (Pl.
I, fig. 3). At 30 days of age, the relative number of fibers present had in-
creased considerably and the first of the vascular parenchyma rays had
formed. At 40 days of age the xylem consisted of radial rows separated
more widely toward the periphery of the stele, and the libriform fibers

had much increased in number. The increase in the xylem of the root continued throughout the growing season. In the latter part of the season, the upper portion of the tap root formed smaller tracheae with fewer fibers interspersed between them. The contrast in size of these small tracheae with the larger ones formed in the spring, made it possible to determine the annual growth, as previously reported by Jones (4).

STRUCTURE OF THE SECONDARY PHLOEM

The first secondary development of the phloem fibers in the root was attributed to the differentiation of cells already present and contiguous to the primary phloem fibers. The secondary phloem formed during at least the first three months of the plant's development continued to provide small groups of fiber initial cells. The large parenchyma cells of the phloem, as they were pushed farther from the cambium, took on the appearance of typical cortical cells. These thin-walled phloem cells, like the ray cells, became filled wtih starch during the storage period of the plant. Interspersed throughout the phloem, fibers comparable in appearance and reactions to the libriform fibers of the xylem were regularly formed in scattered groups.

STRUCTURE OF LIBRIFORM FIBERS

The cells of the libriform fibers varied considerably in size. Those measured ranged from 580 to 2,088 microns in length. The average length of nearly 100 measurements was 1,309 microns. The average thickness taken near the middle of the fibers was 7.1 microns. The fibers were spindle-shaped, the ends being less than half the diameter of the fiber in its middle region. In some fibers the walls were wavy in outline.

A study of the cross-section of the fibers revealed that their walls were composed of primary, secondary, and tertiary layers (Pl. II, fig. 1). The tertiary layer, which varied in thickness according to age and time of year, had a mucilaginous consistency. In the young fibers where the tertiary wall was still thin, nuclei were commonly noticeable. The protoplasm became obscure as the tertiary wall thickened, although the nuclei remained rather conspicuous until the lumina were much reduced. The nucleus of each fiber either became so modified that it was invisible, or disappeared as the tertiary wall thickened, for it could not be seen after the wall reached its maximum thickness. The fibers had simple pits, which connected the lumen of one cell to the lumina of adjacent cells.

FORMATION OF CORK IN THE ROOT

The presence of a cork cambium was first observed in hypocotyls 21 days old and in roots 30 days old. In the roots the small pericylic cells, which gave rise to the periderm, contained dense cytoplasm and nuclei slightly larger than those of the surrounding cells. The cells of the endodermis elongated tangentially and contracted radially as the diameter of the root increased. Before 50 days of age the phellogen manifested little activity except to increase in circumference. In plants 50 days or more of age the phellogen was usually more active and formed several layers of secondary cortical cells and a layer of cork that ranged from three to six layers of cells in thickness at the end of the first season. The secondary

cortex and cork were a relatively small portion of the cross-sectional area of the roots and hypocotyl even in plants several years of age. As growth was renewed each spring, the cork was reduced in thickness by a sloughing of the outer layers and thickened again by the addition of new layers near the end of the season.

THE SLOUGHING OF THE CORTEX AND OTHER SECONDARY MODIFICATIONS

The sloughing of the primary cortex, which is three-fourths or more of the cross-sectional area of the root and hypocotyl in the seedling stage, is a normal procedure in the life of the plant although attended by the danger of admitting organisms if occurring previous to the formation of secondary protective structures. Sometimes sloughing was evident on seedlings five days old, but in other instances there was no evidence of sloughing even on 15-day-old seedlings. A warm temperature suitable for growth seemed to hasten the process. The root cortex of the 10-day-old plants usually showed the outer cortical cells collapsed and fragmentary. The disintegration, beginning with the outer cortical cells, continued inward until all of the cortex was involved. Only fragments of the primary cortex could be found on the roots of 50-day-old plants.

The sloughing of the cortex of the hypocotyl proceeded somewhat differently from that in the majority of the roots in that the breaking down began next to the stele and not in the region of the epidermis. In a large percentage of the plants examined, the cortex of the hypocotyl collapsed only in its inner region, while the outer layers remained intact until finally pushed off by the expanding tissues beneath. The time required for the complete sloughing of the cortex of the root and hypocotyl was approximately the same. In seedlings 50 days of age the cortex had mostly disappeared.

SOME EXTERNAL STRUCTURES

A characteristic feature of the roots and hypocotyls of the older plants is the lenticels. The lenticels occur generally at the place of emergence of the small lateral roots (Pl. I, fig. 2). The lateral roots appear to grow out through the lenticels. Many of the roots, however, appear before the lenticels and likely many of the lenticels are masses of parenchyma which have pushed out through the opening made by the lateral roots. Those new lateral roots which arise directly from the taproot in the early spring, appear just at one side of the old lateral roots and push their way out through the lenticel at that location. In most cases they are branches from the base of the old lateral root. A few lenticels have no apparent connection with lateral roots.

The lenticels are more in evidence both as to size and whiteness on plants taken from a rather moist location. The abundant moisture seems to stimulate the growth of the loose parenchymatous cells, which are characteristic of the lenticels. Under moist conditions the lenticels are white, spongy masses extending tangentially on each side of the lateral root. The lenticels of plants taken from drier soils were similar in shape but smaller, and the color was similar to that of the root.

Another feature of the older alfalfa plants noted was the death and decay of tissues in the central region of the crown. This phenomenon is related to the death of the aerial stems at the end of each season. This

death of tissues was found to extend beyond the aerial stems into the crown, and in old plants penetrated through the hypocotyl into the upper portion of the root. Crowns with such dead centers were still able to continue growth in their peripheral regions. When sufficient live parenchyma remained about the decaying area, it commonly formed a layer of cork that walled off the region of decay. Organisms could enter these areas, and if the dead areas extended radially far enough to invade the xylem, the plants seemed to have difficulty in producing the protective periderm. Under conditions favorable for decay, the dead areas increased in diameter with the age and growth of the crowns. Some three-year-old plants were found with the dead interior regions a centimeter or more in diameter and extending down as far as eight centimeters below the cotyledonary node.

In the spring of 1932 a type of injury caused by cold was noted. There were two late freezes which injured a large percentage of the year-old plants of the plots. The injury was peripheral and started at the cotyledonary node and extended downward on some plants to a depth of five or six centimeters. Examinations of these injured portions showed that a new cork cambium had formed in the secondary phloem. With later development, this brown injured portion was cracked open by the expansion caused by secondary growth, and on freshly dug plants many small lenticels were seen in these open cracks. These lenticels were scattered more or less over the entire surface of the injured portion (Pl. I, fig. 4).

THE CONTRACTION OF THE ROOT AND HYPOCOTYL

The contraction of the root and hypocotyl is a prominent phenomenon in the development of the alfalfa plant the first season. In germination the cotyledons are elevated usually one to two centimeters above the soil, but by the end of the first season the cotyledonary node has been brought below the surface of the soil by the shortening of the hypocotyl and primary root. As has been shown by Jones (4) the contraction of the hypocotyl and root is due primarily to the lateral expansion of parenchyma in the center and throughout the stele of these structures. Studies made by the writer of the hypocotyl and the upper portion of the root disclosed that as the plants continued the production of secondary xylem, there was a decided enlargement of the parenchyma cells in the center of the xylem cylinder. In plants five days old the cells of the central parenchyma were globose with noticeable intercellular spaces between them. These cells later enlarged, became angular and much crowded, a condition quite noitceable by the time the plants were 40 days of age. The central parenchyma cells had an average length of nine, 16 and 21 microns in seedlings with ages respectively five, 15 and 30 days. This expansion seemed to continue during the first year and possibly in some roots into the following year. In plants approximately 13 months old, these central parenchyma cells, measured in transverse sections of the lower region of the crown, averaged 46 microns.

In the upper portion of the taproot of plants one-year-old or older, which once had primary xylem in the center of the root, new parenchyma cells had been formed in the central area apparently by the proliferation of the undifferentiated primary cells. Many of the primary tracheae or groups of them had been widely separated by the enlargement of the

parenchyma interspersed among them. The parenchyma cells adjacent to the tracheae had undergone an extraordinary radial expansion. Some of them in three-year-old plants were as much as 102 microns in radial dimensions, while other types of cells adjacent to them averaged about 60 microns in length and 40 in width. The expansion of the parenchyma in the central portion of the roots brought it in contact with the parenchyma of the rays and thus established a continuity of parenchyma throughout the stele. The cells of the xylem and phloem rays also expanded considerably.

Radial sections through the center of the upper portion of the root and through the hypocotyl showed that the division and radial expansion of the parenchyma cells interspersed among the fibers and tracheae of the xylem separated the xylem into deviating anastomosing strands, which, owing to their sinuosity, were much shortened lengthwise (Pl. I, fig. 3). Tangential sections showed a similar sinuosity of fibers and trachea in the tangential direction, thus disclosing that a tangential as well as a radial expansion of the parenchyma cells had occurred. In the phloem a similar separation of the fiber bundles into sinuous strands was noted. This expansion of parenchyma tissue both radially and tangentially by the enlargement and division of cells accounts for the lengthwise contraction of the hypocotyl and roots. In consequence of the shortening of the non-elastic tissues, the elastic tissue and thus the entire root and hypocotyl were forced to shorten in the same direction, the result of which was the bringing of the crown and other aerial portions nearer or below the soil.

STRUCTURE OF ROOTS WITH ALL TISSUES WELL FORMED

The stele of mature roots comprised many radiating segments of xylem separated by rather wide rays of parenchyma (Pl. II, fig. 2). Each xylem segment was composed of tracheae occurring either singly or in radial rows of two to eight, commonly separated by groups of libriform fibers and xylem parenchyma. In some roots, the radial strands of xylem consisted chiefly of tracheae separated by groups of libriform fibers. The primary cortex, which was usually sloughed during the first two months of the plant's growth, was replaced by a thin layer of secondary cortex and a bark three to six cells in thickness. The phloem was relatively thick and contained much parenchymatous tissue and scattered groups of libriform fibers. The portion of the mature root in the proximity of the hypocotyl varied from the above description in that its central region contained a considerable amount of parenchyma tissue with small patches of tracheae interspersed.

DEVELOPMENT OF THE CROWN

The crown is not a definite morphological structure. In young plants only a month or two of age, the crown may be designated as the upper portion of the hypocotyl plus its outgrowths consisting of first leaf, of the aerial shoots from the buds in the axils of the cotyledons and from the plumule. As the plants increase in age the hypocotyl and root lose their demarcations as a result of their secondary growth, lengthwise contraction, and the prominent secondary growth in the region of the stem bases. In plants a year or more of age, the crown embraces the perennial stem bases now of considerable prominence in size and the portion of the root-

like axis which now includes the hypocotyl and at least the upper part of the root.

The first or unifoliate leaf developed independently of the plumular meristem. Its primordium was present in the embryo and was formed approximately at the time the primordium of the plumule was formed. The other leaves were products of the plumular meristem.

The position of the second leaf trace was opposite that of the unifoliate leaf period. The position of the third leaf was about 135° clockwise to that of the second leaf and the fourth leaf was opposite the second. The later phyllotaxy was 4/1.

DISCUSSION AND SUMMARY

The investigations reported in this article sustain the following statements concerning a number of the anatomical features of *Medicago sativa*.

The embryo of *Medicago sativa* has two meristems at the plumular end of the hoypocotyl. The one apically located is the plumular meristem. The other one, which is a little to one side of the plumular meristem, is the primordium of the first leaf.

The stele of the primary root is prevailingly a triarch protostele as reported by van Tieghem (8), de Bary (1) and Winter (9). Four vascular plates in the upper part of the young primary root were occasionally noted, one of which was less developed than the others. This tetrarch condition has been reported by van Tieghem and Winter. The tetrad condition in the upper part of the young primary root is apparently caused by the extension into the root of one of the bundles of the hypocotyl, a conjecture supported by the fact that tetrarchy becomes more prevalent in the upper portion of the primary root as the seedlings increase in age. The stele of the secondary roots is generally diarch. The protostelic condition of the primary root extends several millimeters into the hypocotyl before a separation of the vascular strands by parenchyma occurs. The stele of the hypocotyl is tetrarch.

The primary vascular system of the root and hypocotyl terminates in the cotyledons. The vascular system of the root, hypocotyl, and cotyledons constitute a complete and independent system. The vascular system of the first leaf and of the shoots from the plumule is subsequently superimposed upon the primary bundles in the hypocotyl. The observation in regard to the independent development of the two vascular systems accords wtih Winter's report but not with van Tieghem's observations that one of the three primary strands of the primary root passes into the first leaf. According to Compton (2), the independent development of the two vascular systems is characteristic of epigeal legume seedlings.

The libriform fibers which occupy a prominent place in the stele of the root and hypocotyl appeared in seedlings about 20 days of age and were formed throughout the growth of the plants in groups interspersed through both the xylem and phloem. The walls of the libriform fibers consist of three layers, the inner one of which is pliable and generally becomes very much thickened.

The phellogen in the root and hypocotyl arises in the pericycle, an observation at variance with that of Jones (4), that the origin of the phellogen in the stem bases of alfalfa is in the endodermis. The phellogen was rather inactive until the plants were almost two months of age. During

the first season it formed several layers of secondary cortical cells and a layer of cork ranging three to six cells in thickness. The secondary cortex and cork when at their maximum development occupy a relatively small peripheral portion of the cross-sectional area of the root and of the hypocotyl.

The sloughing of the primary cortex of both the primary root and hypocotyl was usually detectable in plants 10 to 15 days of age and was usually completed or nearly so in plants 50 days of age.

As previously reported by Jones (4), the lengthwise contraction of the root and hypocotyl of alfalfa is the result of the expansion of parenchyma in the center of the stele and interspersed among its vascular elements. The expansion of the parenchyma both radially and tangentially separates the vascular strands of the stele and forces them into sinuous courses, resulting in the shortening of the strands, which in turn compresses the other tissues and results in a general contraction.

Lenticels are rather prominent structures on the taproots of alfalfa, especially at the end of the season's growth. They are masses of callus tissue which are generally about the bases of the lateral roots and appear in most cases to have been produced in response to the wound stimulus accompanying the emergence of the lateral root.

The death of the tissues in the interior of the crown may not always be attributable to mechanical injuries or to the invasion of organisms. It was common in plants undisturbed by mowing or pasturing. It was associated with the decay started in dead bases of the shoots and may be in part a natural consequence of the passing of the interior tissues into an inactive state, as in the case of many trees in which the heart-wood decays after its functions are taken over by exterior layers of tissue.

Acknowledgments are due Dr. J. N. Martin, under whose dierction the work was done, and to Dr. J .E. Sass for helpful suggestions.

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EXPLANATION OF THE PLATES

PLATE I

Fig. 1. A series of young alfalfa plants showing the early developmental features.

Fig. 2. Alfalfa roots showing the location of the lenticels. The top of the left picture shows more plainly the three-ranked arrangement of the secondary shoots.

Fig. 3. Photomicrograph showing the sinuate condition of the fiber groups and the tracheae. Cr., some crystals adjacent to a fiber strand.

Fig. 4. A winter injured root which shows new lenticels in the cracked open dead portion.

Fig. 5. Photograph of the vascular system of a 24-day-old plant.

Fig. 6. Photograph on left of the vascular system of the second leaf petiole base and attached stipules. Picture at the right shows the vascular connections to the three leaflets of the same leaf.

Fig. 7. A transverse section through a crown branch showing the pith, the lignified cylinder and the small fiber groups in the "bark."

Fig. 8. A portion of a lenticel on a three-year-old alfalfa root.

PLATE I
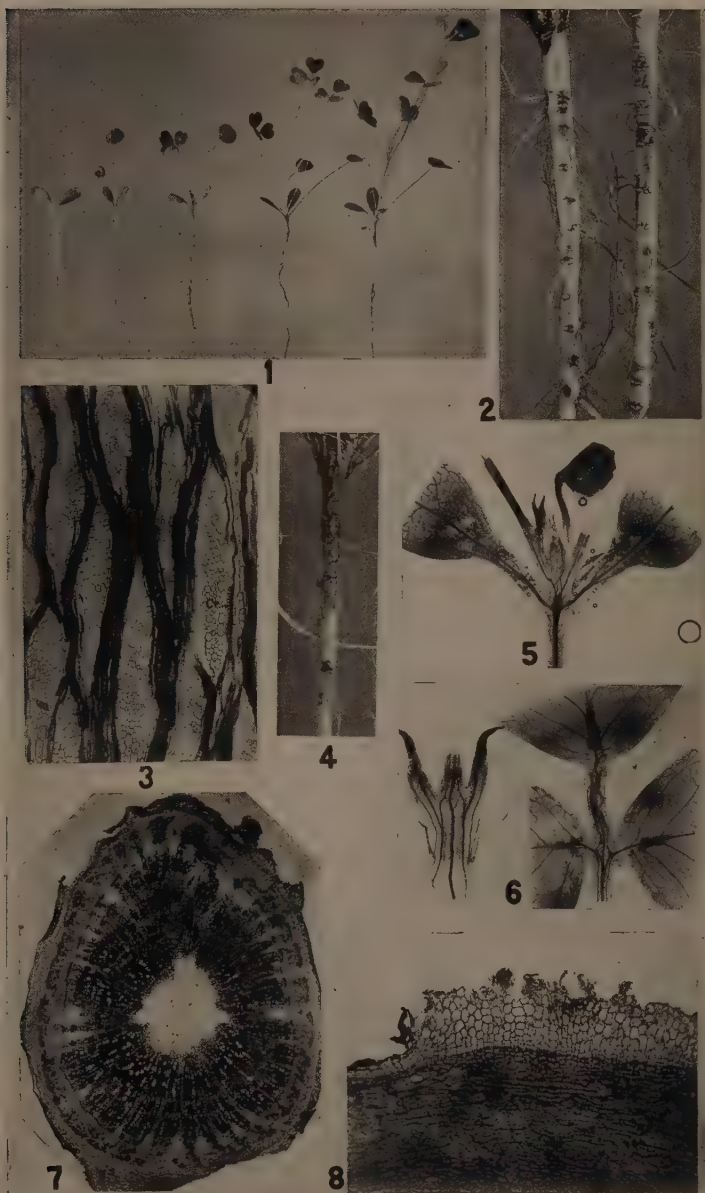
PLATE II

Fig. 1. Photomicrograph showing the two layers of the libriform fiber walls. The first and second layers show as one layer.

Fig. 2. A section of an alfalfa root about 13 months old, showing the thin layer of cork present during the growing season.

Fig. 3. Section of upper taproot with a dead center. Note the phellogen (P) formed around each of the dead areas.

Fig. 4. The vascular system of a five-day-old plant showing the two bundles leading into each coteyledon (C).

Fog. 5. Section of a root 50 days old. Note the triarch arrangement of vessels in the center of the section and also the last remains of the primary cortex clinging to the outside.

PLATE II
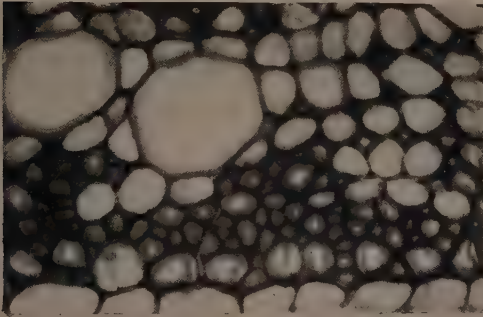
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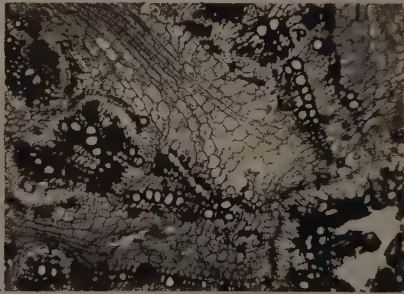
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ALEGRIA—A POPPING SEED USED IN MEXICO AS A SUBSTITUTE FOR POP CORN (AMARANTHUS CAUDATUS L. VAR. LEUCOSPERMUS TH.)[1]

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While in Mexico the past winter the writer took occasion to visit a number of the public markets in typically native regions to ascertain what types of pop corn were used. We were surprised to find no pop corn, though parched field corn was universal. In the place of pop corn, a popped seed termed Alegria was offered as a confection. Unpopped seeds appeared to be an Amaranthus. Through the courtesy of Prof. C. Conzatti we secured specimens of the plant, which was determined as *Amaranthus caudatus* L. var. *leucospermus* Th. Seed from these plants were sown the middle of September in the college greenhouses. The plants grew readily and developed to full bloom within two months. In general appearance the plant resembles the purple Amaranthus, *Amaranthus paniculatus,* which is grown for its foliage. The foliage and blossoms are shown in Plate I, figure A.

The Alegria possesses angular stems, many of which are deeply tinged wtih crimson, as is also the under surface of the leaves. The flower spikes, though not as long and slender as in *A. paniculatus,* possess a deep crimson cast on many—though not on all—of the plants, suggesting its possible value as an ornamental as well as a food plant.

The seed of Alegria are of a light buff color, circular, approximately one millimeter wide, half as thick, and quite uniform in size. The seeds are distinctively hyaline and very hard. The funiculus is prominent and bears a deep furrow which carries a red line. As will be noted in Plate II, figure C, the cells are packed with starch grains. The starch grains are small and quite uniform as to size. These are shown in Plate II, figure A.

Measurements were made to determine the volumetric increase caused by popping in comparison with pop corn. Alegria increased 1050 per cent, while the Japanese Hulless, a leading commercial variety of pop corn, gave an increase of 1040 per cent. From this standpoint Alegria compares favorably with pop corn. The relative size of the unpopped and popped Alegria seed is indicated in Plate II, figure B.

The popped Alegria seed is of a snow-white color. The confection is usually sold on the market in the form of a small, rectangular block, honey being used as a binder. Apparently the caramelization of the honey gives the confection a brownish cast. Because of its color, the hull is inconspicuous in the finished product. A block of the Alegria confection is shown in Plate I, figure B.

The Alegria is probably an ancient food plant of the Indian. Safford[2]

[2] Safford, W. E. 1915. A forgotten cereal of ancient America. Int. Cong. of Americanists, 19: 286.

identified this plant associated with the huauhtli ceremonies of the Aztecs, and it is generally recognized that religious expressions are one of the most conservative elements of a people and the least likely to take on anything new.

Alegria appears to be indigenous throughout the temperate zones of Mexico and extends northward to the bordering region of the United States. In the state of Oaxaca, in southeastern Mexico, we observed small patches which were being cultivated for the seed crop. Several species of Amaranthus thrive and fruit abundantly in this state. Alegria might have a possible value as a breakfast food in competition with puffed cereals, for which the American palate shows a liking.

PLATE I

Fig. A. Alegria. *Amaranthus caudatus* L. var. *leucospermus.*

Fig. B—Alegria confection.

PLATE I

PLATE II

Fig. A.   The starch grains of Alegria are very small and of a uniform size. (350X.)

Fig. B.   Popped and unpopped Alegria seed. The light tan color of the seed coat, instead of black as in most species of Amaranthus, is an important advantage for popping. (5X.)

Fig. C.   The cells of the Alegria seed are densely packed with starch grains. (350X.) (Photo by courtesy of Dr. J. N. Martin.)

PLATE II
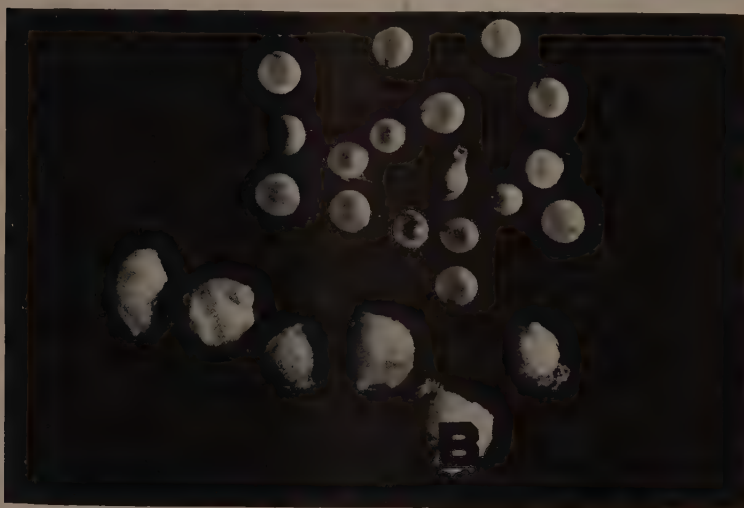
A PRELIMINARY REPORT ON THE ANATOMICAL STUDY OF AN INHERITED EYE DEFECT IN THE GUINEA PIG

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In 1932 Lambert and Shrigley (2) presented a report before the Iowa Academy of Science on an inherited eye defect which had been observed in the colony of guinea pigs maintained in the genetics laboratory at Iowa State College. The defect is apparently due to a single Mendelian recessive gene, although there is some evidence that modifying factors affect the degree of expression. Since that time preliminary study has been made on the tissue changes associated with the defect, the results of which are incorporated in this paper.

Because of the extensive material written on hereditary eye defects and because most of it is not available at the present time to the author, no attempt will be made to review the literature. A cursory examination has, however, revealed no reports of any similar condition.

METHODS AND MATERIALS

The defective eyes were obtained from guinea pigs in the colony showing the defect. Normal eyes for the purpose of comparison were obtained from four unrelated sources.

In order to determine the exact part of the eye involved it was necessary to develop and use a celloidin technique. We had only mediocre success following closely the use of methods developed by technicians in other laboratories. This was probably due to differences in materials used and working conditions. Then too, the embedding of such small eyes presented special problems. We have, however, come to the conclusion that good sections can be obtained with any standard celloidin technique as soon as one has had enough experience to tell when the thickening of the celloidin solution used for embedding has progressed far enough. The technique that gave the best and most consistent results is a combination of the techniques used by Miss McLaughlin at the clinic of the Mayo Foundation at Rochester, Minnesota (5), and Walls (9). The fixing solution used was the formalin, acetic-acid-Zenker's solution recommended by Meeker and Cook (7).

Small embedding boats made of fiber blocks and strips of paper were found to be convenient and practical aids in the embedding of small eyes. When the embedding solution had reached the proper viscosity the eye was removed from it and placed in a boat in such a manner that the desired plane of sectioning lay parallel with the fiber block which formed the bottom of the boat. Then the boat was immediately filled with the solution from which the eye was taken and the whole immersed in 80 per cent alcohol for hardening. Just before the blocks were ready to section the paper around the outside was peeled off.

The boats referred to are made from three-fourths inch fiber cubes and paper in the following manner: A piece of paper three and three-fourths by two and three-fourths inches is painted along one edge with a three-fourths inch band of weak celloidin. Then, after placing the fiber block at one corner, the paper is folded about the block in such a manner as to form a prism with the block as the base. The free edge of the paper is also glued with celloidin, and the whole is immersed in chloroform to harden. If the boat so formed is not yet water-tight, more celloidin may be painted around the leaking spots.

Sectioning was done in the customary manner using a sliding microtome and keeping the block wet with 80 per cent alcohol. The sections were preserved in 80 per cent alcohol until wanted. Just before staining the mercury crystals left from the fixing solution were removed by leaving the sections in a weak iodine solution in 80 per cent alcohol for twelve to twenty-four hours.

Two methods of staining were used. The first was an ordinary hematoxylin-eosin staining technique. The other method [Sloss (8)] was tried in the hope of differentiating the ciliary muscle of the eye from the surrounding structures. This did not prove practicable, but such beautifully stained sections were obtained that we believe the technique worthy of mention. The individual stains used in this technique have long been used in ophthalmology. However, we have not been able to find any record of their being used in combination in this particular field. By use of this technique each part of the eye is very clearly differentiated, thus making it especially valuable for student instruction. The layers of the retina, the posterior homogeneous membrane of the cornea (there is no anterior homogeneous membrane in the guinea pig cornea) and the corneo-scleral junction particularly, are well demonstrated. The stains color the cornea and sclera differently. Time did not permit extensive experimentation with this staining technique but results were so good that we believe it worthy of special work to determine its possibilities in the ophthalmologic field.

1. 80 per cent alcohol—two changes
2. Distilled water
3. Delafield's hematoxylin—3 to 5 minutes
4. Tap water
5. Weigert's elastic connective tissue stain—1 hour
6. Wash in distilled water
7. Van Gieson's stain—5 minutes
8. 80 per cent alcohol
9. Oil of origanum—until cleared
10. Xylol—No. 1, 5 minutes
         No. 2, mount from
    (To 8, 9 and 10 add a few crystals of picric acid)
11. Mount from xylol No. 2 into Canada balsam

After definitely determining that the trouble was confined to the cornea, the corneae were removed from the eyes to be studied and dehydrated and then embedded by the rapid, acetone-paraffin method [McNamara (6)]. Serial sections were mounted in balsam after staining by a routine, hematoxylin-eosin technique.

Later a method of injecting the cornea with an ordinary carmine-gelatine mass was developed, and the whole cornea was mounted in balsam. For the injection we followed, with a few minor modifications, the method described by Mann (3). After the injection had been completed the whole animal body was immersed in a 10 per cent formalin solution for twenty-four hours. At the end of this time the anterior half of the eye was sliced off with a razor and placed in 10 per cent formalin for from two to twenty-four hours.

The clearing method used was developed by Dr. Becker and Mr. Roudabush of the Zoology Department, Iowa State College, and modified to suit our needs.

70 per cent alcohol
80 per cent alcohol
Two-thirds of 80 per cent alcohol, one-third of aniline solution
One-third of 95 per cent alcohol, two-thirds of aniline solution
Aniline
One-half aniline and one-half oil of wintergreen solution
Oil of wintergreen
(Leave in each solution for fifteen minutes after the tissues sink.)

Then, keeping the tissue wet with oil of wintergreen, the lens is picked out with a needle and the iris and ciliary body peeled out with a blunt-nosed forceps. Most of the sclera is trimmed off, leaving just enough to allow easy recognition of the corneo-scleral margin. The tissue now closely resembles a watchglass. This is placed upon filter paper and the oil is drained off. Meanwhile coverglass supports, which will allow the coverglass to just clear the tissue, are placed upon the slide. These are advantageously made of chips of a thick glass slide. Two layers usually suffice for height and four supports are enough. Then the tissue is grasped by the edge with a pair of forceps and holding it at an acute angle with the slide in the center of the four supports with the concave side down, balsam is run under it with a pipette, and as the air is displaced from under the tissue, it is released and a coverglass is placed upon the supports. The rest of the space between the coverglass and the slide is filled with balsam from the pipette. Although the mount can be observed at once if one is careful, it is best to wait several days to allow the balsam to partially harden.

THE GUINEA PIG CORNEA

The guinea pig cornea does not differ markedly from the cornea of other mammalia. The epithelium is stratified squamous in type and consists of a single, basal row of columnar cells, three or four intermediate rows of polygonal cells, and three or four superficial layers of squamous cells. The epithelium is thrown into a fold at the margin of the cornea and returns to its normal thickness over the sclera and palpebra where it is continued as scleral and palpebral conjunctiva. Just inside of the margin of the cornea there begins a deeply pigmented area, which is continued out into the scleral conjunctiva. The area of pigmentation is very regular and distinct and can be easily traced both in whole mounts and sections (fig. 1). This pigmentation is due to the presence of numerous

stellate, pigmented cells in the columnar cell layer of the conjunctiva.

There is no anterior, homogeneous membrane in the guinea pig cornea. Its place is taken by a thin, basement membrane.

The propria of the cornea of the guinea pig is similar to that of any mammalian cornea. The fiber bundles are laid in regular rows. Here and there are scattered corneal corpuscles. It is well to emphasize here that the normal cornea is an avascular structure. The only blood vessels present in it are tiny, capillary loops which extend for a very short distance into the cornea from the corneo-scleral junction (fig. 1). These are very regular in extent.

The posterior homogeneous membrane and the endothelium are worthy of no special attention.

SYMPTOMS OF THE DEFECT

The symptoms of the defect are extremely variable in intensity. Some animals are apparently normal in one eye while the other eye is severely affected. Some few animals show both eyes to be apparently normal, although they come from the affected stock and have litter mates with very severe involvement of both eyes. None of these apparently normal eyes from affected stock have as yet been available for histologic study.

The most common symptoms observed are a dullness and drying of the cornea, photophobia, some loss of tactile sense, and thickening of the eyelids, which may or may not be accompanied by a small amount of purulent exudate. Ulceration is occasionally observed and keratinization may progress so far as to give the cornea the appearance of a callus. With the exception of the eyes showing ulceration and keratinization, there is evidenced no true opacity of the cornea. Comparative examinations with the ophthalmoscope of normal and abnormal animals reveal that the dullness of the cornea of the latter animals merely prevents complete examination of the fundus of the eye without providing an opacity that can be focused on. An interesting fact disclosed by these examinations, which were made by Dr. Dyer, was that the guinea pig has a very poor set of eyes normally. All of the animals examined were extremely myopic and some apparently normal animals showed retinal degenerations which resembled those of syphilis in man.

The apparent micro-ophthalmia observed by Lambert and Shrigley (2) is due largely to a thickening of the eyelid and conjunctiva plus the retraction of the eye whenever it is exposed to a light that is at all strong (Photophobia).

THE TISSUE CHANGES

The condition is characterized by a vascularization of the cornea accompanied by a variable amount of melanosis and other tissue changes in the propria and epithelium. So far there has been observed no evidence of tissue change in the posterior homogeneous membrane or in the endothelium. The blood vessels may appear merely as an increase of the normal vessel loops of the cornea, and be fairly regular in outline although abnormal in extent; or they may be so large as to be visible to the naked eye in injected specimens showing many branches all through the cornea.

The blood vessels are located in the propria usually near the epithelium. Sometimes, however, a few branches may appear in the deeper layers of the propria.

The melanosis is quite variable in extent and incidence. It may appear in the form of large sworls across the surface of the cornea (fig. 2) or it may apparently be absent. There may be only a few isolated pigment cells some distance from the pigment border. Sometimes the melanosis is only manifested by an abnormally ragged and irregular line of demarcation between the pigmented and non-pigmented portion of the cornea. In the abnormal eyes this line is quite often not as distinct and black as in the normal eyes. The abnormal melanosis, like the normal corneal pigmentation, is due to the presence of stellate pigment cells in the deeper layers of the epithelium (fig. 4).

The epithelium is especially prone to show changes in eyes affected with this condition. Some corneae show a simple proliferation of the epithelium and loss of definite stratification (fig. 4). In these corneae the epithelium is largely composed of polygonal cells. Varying degrees of keratinization are also observed. This may be so extensive that it causes the cornea to take on the appearance of a callus. The basement membrane is occasionally thickened over the vascular area. Sometimes it may appear to be absent in small localized areas allowing the epithelium to rest directly upon the propria. The folds of epithelium at the limbus are often thickened and accentuated. The epithelium may, however, show no lesions at all. Ulcers and erosions have been observed both macroscopically and microscopically. The epithelial proliferation and keratinization may involve the whole cornea or be localized.

Changes in the propria are also common. Often the fiber bundles of the affected propria appear to lose their identity, and the propria take on the appearance of areolar connective tissue. Again there may be a small amount of scar tissue laid down directly under the epithelium. Some propriae show no changes apart from the vascularization. Leucocytes may be present in varying amounts. Often a number of phagocytic cells quite distinct from the ordinary blood phagocytes are present. It is not clear as to whether these are corneal corpuscles or are of endothelial origin. Lymphocytic infiltration, a common manifestation of chronic conjunctivitis, is often noted under the scleral and palpebral conjunctiva. It is especially liable to occur at the corneo-scleral junction.

DISCUSSION

With the exception of the melanosis, all of the lesions of this disorder are those of inflammation and at first glance it would appear that the primary etiological factor would be an inflammatory agent of some sort. Of course, before any definite conclusions as to the etiology of this condition can be made, it will be necessary for extensive work to be done on the development of the lesions in the fetus because the guinea pig born with this defect usually carries it throughout its life with little or no variation in intensity. Work will also have to be done to determine whether or not the partial loss of tactile sense in the affected cornea is due to changes in the corneal nerves.

It is our opinion that this condition is not primarily due to an inflam-

matory agent. First, because of the tendency of the affected eyes to show little or no change in extent or severity of lesions from birth to death; second, because of the genetic behavior of the condition; third, because the amount of vascularization is never commensurate with other evidences of past or present inflammation; fourth, because inflammation will not account for the melanosis of the cornea.

We have at the present time two theories to explain the condition. The first considers that the condition is primarily an aberration in development which interferes with the normal nutrition of the cornea and that the vascularization is compensatory. This disturbance of nutrition also causes the nerves of the cornea to lose some of their sensitiveness. Hypersensitivity of the cornea is a protective mechanism for this delicate structure, and as it is impaired in this case, the cornea is subject to irritation and injury from a number of sources. This would account for the inflammatory changes in the eyelid and anterior parts of the cornea. According to Collins and Mayou (1), photophobia is commonly observed in derangements of the cornea and in this case it is only important in that it may be partially responsible for the thickening of the eyelids and conjunctiva as it results in the eyelids being used to an abnormal extent and thus causing mechanical irritation. The chief evidence supporting this theory lies in the fact that even in the normal guinea pig eye it is difficult, if not impossible, to demonstrate a Canal of Schlemm. This canal is supposed to be an important factor in the nutrition of the cornea and its absence would indicate that even at best the guinea pig cornea is not as effectively nourished as that of other animals. Thus the way is paved for a slight aberration in development to interfere with the normal lymphatic nutrition of the cornea and bring about a compensatory vascularization. It is to be noted, however, that this theory does not account for the melanosis.

Mann [in the section on the histology of the eye in "Special Cytology" edited by Cowdry (4)] advances the opinion that the transparency of the cornea and lens instead of being a special modification of these tissues is due to a retention of the normal transparency of the embryo by these structures. The evidence she submits is so conclusive that we are ready to accept this theory as fact and upon it base our second theory. This attributes the condition to an inherited tendency on the part of the cornea to continue on and assume some of the properties of the skin instead of remaining arrested in its normal state. With the blood vessels in the propria, the melanotic cells located in the basal cell layer of the epithelium, the loss of hyperesthesia of the tissue, the keratinization and epithelial proliferation observed at birth, and the degenerative changes in the propria which cause it to resemble areolar connective tissue, the cornea presents a structure which appears very much like that of the cutis and subcutis. The fact that the corneal epithelium and the epithelium of the skin both originate from similar ectoderm and that the subcutaneous connective tissue and the tunica propria of the eye both come from sub-ectodermal mesoderm renders this theory still more tenable. The other changes, i. e., post-natal keratinization and epithelial proliferation, leucocytosis, localized lymphocytosis, connective tissue proliferation, ulceration, and so forth, can all be ascribed to inflammation which is the result of irritation, injury and infection of these delicate structures which are no

longer protected by their normal hyperesthesia. Not to be disregarded in this respect is the irritation produced by the constant use of the eyelids in an attempt to keep light out of the photophobic eye.

SUMMARY

(1) An inherited eye defect in the guinea pig which is apparently due to a single Mendelian recessive gene was studied grossly and microscopically.

(2) Methods of embedding small eyes, of staining eye sections, of mounting injected corneae were developed.

(3) The symptoms of the condition were dullness and drying of the cornea, photophobia, some loss of tactile sense which may or may not be accompanied by a purulent exudate. Ulceration and keratinization also were observed.

(4) Microscopically the condition is an abnormal vascularization of the cornea accompanied by a variable amount of melanosis and inflammatory changes in the propria and epithelium of the cornea.

(5) Two working hypotheses are advanced in an effort to explain the condition.

ACKNOWLEDGMENTS

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PLATE I

Fig. 1. Corneo-scleral margin of normal guinea pig eye (X150)
A. Line of pigmentation
B. Capillary loop

Fig. 2. Injected whole mount of defective guinea pig cornea (X7)
A. Abnormal melanotic area
(The fine lines are all blood vessels)

Fig. 3. Section of normal cornea (X800)

Fig. 4. Section of defective cornea (X800)
A. Pigmented cells

PLATE I

Fig. 1
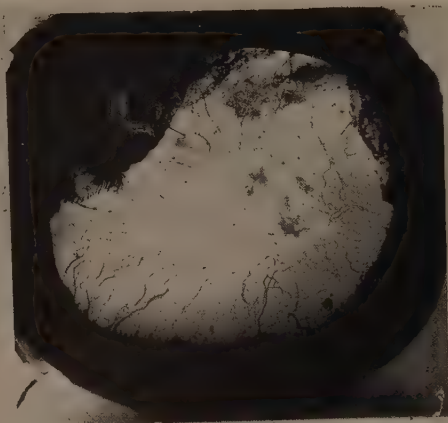
Fig. 2

Fig. 3

Fig. 4

INTRACELLULAR ABNORMALITIES ASSOCIATED WITH YELLOW DWARF OF ONIONS[1]

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The study with which this paper is concerned was conducted in conjunction with investigations of the relationship of insects as vectors of a virus diseases of onion, known as yellow dwarf, which have been previously published in part by Drake, Tate and Harris (1932, 1933). In considering the possibilities of this problem, it was hoped that a fairyl complete cytological study of the virus-affected tissue could be conducted, but insufficient time has made it necessary to confine efforts to a more limited field, that is, to a determination of the presence of intracellular bodies. In a subsequent paper will be published the results of studies concerning the effect of feeding punctures of aphids on certain plant tissues.

The presence of cell inclusions in plant tissue affected with a virus disease was first reported by Iwanowski in 1903 in connection with a study of tobacco mosaic. Since that time many workers have made contributions to this phase of the plant virus problem, the reports of which are accompanied in some cases (Goldstein, Smith, Cook) by elaborate literature reviews. As a result, it seems desirable at this time to review briefly only a few of the publications which are closely related to the present problem and pertain particularly to monocotyledonous plants.

In 1910 Lyon described intracellular bodies in sugar cane tissues affected with Fiji disease. These structures were later described in detail by Kunkel (1924). Kunkel (1921) in discussing the causative agent of corn mosaic described irregularly shaped bodies which were closely associated with the host cell nucleus and were believed to be living organisms. About a year later Kunkel (1922) reported somewhat similar bodies in mosaic diseased tissue of *Hippeastrum equestre* and of sugar cane. McKinney, Eckerson and Webb (1923, 1924) reported that irregularly shaped intracellular bodies were associated with mosaic in *Hippeastrum Johnsonii* and with rosette and mosaic in wheat. Cook (1925) found intracellular bodies in sugar cane affected with mosaic but they were by no means abundant or conspicuous and occasionally they were entirely absent in severely diseased plants. In some cells in which there were peculiar bodies no nuclei were recognizable. In some cells of both diseased and apparently healthy plants he found two or more nuclei present. Eckerson (1926) reported the presence of intracellular bodies resembling flagellates in mosaic wheat and *Hippeastrum Johnsonii*. Sheffield (1934) has suc-

ceeded in inducing the early symptoms of mosaic by treating healthy tissues with certain protoplasmic coagulating substances. By treatment of healthy tissues with molybdic acid he induced the formation of cytoplasmic bodies similar to the intracellular bodies in tissues affected by Aucuba mosaic.

MATERIALS AND METHODS

The investigations were confined to the intracellular bodies associated with the yellow dwarf disease of the onion and involved a comparison of the protoplasts of diseased and healthy plants. The material for examination was selected from plants grown in both field and greenhouse, some of which had been experimentally inoculated by means of aphids while others had become infected under natural conditions in the field. Some of the diseased plants had carried the infection through three seasons of growth, whereas others had been infected recently. Also, material was included from plants in different stages of development.

Studies were made of both fresh and fixed material. The living material was sectioned free-hand. Some of the sections of living material were mounted in water and examined without further preparation, whereas other sections were killed, stained, and mounted in glycerol.

The killing solutions tried were chromacetic, Bouin's, and the alcohol-formalin-acetic acid solution. These solutions were used in combination with a number of stains—namely, hematoxylin, safranin, gentian violet, analine blue, and haemalum. The chromacetic fixing solution (one part of one per cent chromic acid plus one part of one per cent acetic acid) and haemalum made the best combination. Both paraffin and free-hand sections fixed and stained as described above were employed, but free-hand sections were more satisfactory.

The observations and also the photomicrographs of the intracellular bodies were made with a Spencer microscope equipped with oil immersion and 4 mm. objectives and a 12X ocular.

DESCRIPTION OF THE INTRACELLULAR BODIES

As a general rule the intracelludar bodies were rather sparse even in the onions exhibiting extremely severe macroscopic symptoms. Only occasionally were more than three or four bodies present in the field of the oil immersion objective. They were also very irregularly distributed, being entirely absent in some areas and present in considerable numbers in other areas of the same section. The number of intracellular bodies present in a cell was usually not more than one or two (figs. 6, 8, 9), but occasionally four or five, in which case they were commonly in a close group (fig. 5). Apparently the intracellular bodies in onions infected with the yellow dwarf virus are not so numerous as has been reported in sugar cane, corn, and some other plants having a mosaic disease.

The intracellular inclusions varied much in size, shape, structural appearance, and in their position with reference to the nucleus and to each other. They were usually similar to the nuclei in size and shape (figs. 6, 8). Often they could not be distinguished from nuclei with certainty (fig. 4). Some were much larger than the nucleus (figs. 2, 3). Usually

they were more vacuolate and took less of the stain than the nuclei (figs. 3, 10), but often in structural features and in their behavior with reference to stains they were indistiguishable from nuclei (figs. 6, 8). They were nearly always either close to or in contact with the nucleus, either directly (figs. 3, 6, 9, 10) or by connections of similar material (figs. 4, 8). Sometimes they partially surrounded the nucleus as shown in figure 2. Where several were present in the same cell they were usually in a close group which was either close to or in contact with the nucleus (figs. 5, 7, 11). The members of the group were usually in direct contact but occasionally they were joined by narrow connections (fig. 4).

In comparing material from apparently healthy and diseased plants the difference in respect to intracellular bodies was not so clear cut and striking as was anticipated, for cells were occasionally found in the apparently healthy tissues which were either multinucleate or possessed bodies similar to some of the extra bodies in the diseased plants. They deviated from the usual condition of the healthy tissue shown in figure 1. The cells with extra bodies were much less frequent and less variable in the healthy plants, but it was not impossible to find in the supposedly healthy tissues duplicates of a number of the types of intracellular bodies present in the diseased tissues.

DISCUSSION

No definite conclusions were made concerning the intracellular bodies in the onion. At least two features suggested that they were of nuclear origin—namely, their frequent contact with or close proximity to the nuclei and the fact that they were often very similar to nuclei in form, structure, and in reaction to stains. In fact, sometimes they were indistinguishable from nuclei. Such bodies as shown in figures 6, 8, 9 and 10 may be interpreted either as nuclei in the process of amitotic division or as a nucleus and an intracellular body in close contact. The clusters of bodies in figures 5, 7 and 11 may be the result of repeated nuclear division and differences in the subsequent growth or degeneration of the daughter nuclei.

It is possible that the onions selected as representatives of healthy plants were not entirely free from the yellow dwarf virus but did not manifest the ordinary recognizable symptoms. If these onions were healthy then the presence occasionally of cells which were evidently multinucleate would indicate a tendency of onion cells toward the multinucleate conditions. The presence of a virus in the protoplast of the cell would probably enhance the tendency of the nucleus to divide and also cause abnormalities in the form, size, and structure of the nuclei.

There is also the possibility that the intracellular bodies are formed from the cytoplasm in response to the effect of the virus. Sheffield's (1934) work in which he induced the formation of bodies in the cytoplasm of healthy tissues by the use of chemicals makes such an explanation tenable. That the intracellular bodies in the onion are results of the action of the virus on the protoplasm is more in accord with the nature of the bodies than the theory that they are organisms or substances that cause the disease.

SUMMARY

Intracellular bodies were found in the tissues of onions affected with the yellow dwarf virus.

The intracellular bodies were not numerous and were very irregularly distributed.

They commonly resembled nuclei but varied much in size, form, and structure.

They were usually either in contact or close to the nuclei.

Their position wtih reference to the nucleus of the cell and their frequent close similarity to nuclei suggested that they were of nuclear origin, possibly through amitotic nuclear division.

In tissues from apparently healthy plants, cells that were evidently multinucleated were found occasionally and some cells were observed in which bodies were present that differed somewhat from typical nuclei and that were similar to some of the types of intracellular bodies in the diseased onions.

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PLATE I

EXPLANATION OF PLATE
ONION YELLOW DWARF

All magnification X 300–400

Fig. 1. Cross section showing nuclei of healthy tissue.
Fig. 2. A large intracellular body partially surrounding the nucleus.
Fig. 3. A large foreign body closely applied to the host cell nucleus.
Fig. 4. Three closely associated intracellular bodies of which the darker one is prob-
          ably the nucleus.
Fig. 5. A large, deeply staining amoeboid mass in which it is impossible to identify
          the nucleus.
Fig. 6. Section showing the relative distribution of the abnormal structures of
          chlorotic tissue.
Fig. 7. A large intracellular body completely separated from the host cell nucleus.
Fig. 8. An intracellular body and nucleus connected by protoplasmic strand.
Fig. 9. An intracellular body and nucleus joined by broad connection.
Fig. 10. An enlarged body less intensely stained than the nucleus.
Fig. 11. Three closely associated intracellular bodies of which the exact nature and
          relationship is not known.

PLATE I

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